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BRUCELLA ABORTUS INFECTION OF GOATS

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SUSCEPTIBILITY OF GOATS TO *Br. Abortus.*

IN view of the predominant part played by the goat in the epidemiology of *Br. melitensis* infection, and the close relationship between that organism and *Br. abortus,* it is surprising that more work has not been carried out on *Br. abortus* infection of goats.

Parisot, Vidal and Levy (1932), Giles, Peres and Culty (1932), Taylor, Lisbonne and Roman (1932) have recorded that cows, the natural host of *Br. abortus,* may become naturally infected with *Br. melitensis* and excrete the organism in the milk.

Taylor, Vidal and Roman (1934) showed that *melitensis* may be excreted in the milk of naturally infected cows for periods varying up to 18 months.

There appears to be little information available regarding the susceptibility of goats to natural infection with *Br. abortus,* although some work has been carried out on their artificial infection.

Zwick and Zeller (1913) inoculated five pregnant goats, two intravenously and three *per os,* with heavy doses of a bovine strain of *Br. abortus.* Those inoculated intravenously aborted on the 41st and 59th day, respectively. Two of the three goats infected *per os* kidded normally, and the third aborted 59 days after the first infective dose ; this goat was given on eleven successive days the growth from an agar slope.

Three control goats were kept in contact with the infected goats ; two of these remained negative to the agglutination test, while the third showed a titre of 1 : 40 which fell in the course of a month to 1 : 10.

Burnet (1928) carried out comparative experiments on goats with recently isolated strains of *Br. melitensis* and *Br. abortus.* One goat (Vod) received nine instillations (dose?) of *abortus* into both eyes over a period of six weeks, but failed to develop specific agglutinins in the blood.

Abortion was induced in three goats artificially infected, two intravenously and one subcutaneously, with *Br. abortus*.

Van der Hoeden (1933) infected an adult he-goat by instilling into the conjunctival sac 10 drops of *Br. abortus* suspension. On the 7th day the serum gave positive reactions to agglutination and complement-fixation tests. Positive blood cultures were obtained on the 3rd, 4th and 11th days after the infective dose. The animal was autopsied on the 35th day and *Br. abortus* isolated from the submaxillary lymphatic glands and spleen.

The Susceptibility of Pregnant Goats to Artificial Infection with *Br. abortus*.

A serious obstacle to large scale experiments on *Br. abortus* infection of bovines is the heavy cost involved. The small number of animals that can normally be used in experiments retards progress and reduces considerably the value of the results obtained.

As far as information is available no small laboratory animal appears to be of any value for the elucidation of many problems which still remain unsolved in connection with different phases of brucella infection.

The present inquiry was carried out on the suggestion of the Director of the Laboratory, Dr. W. H. Andrews, in an attempt to add to the rather meagre information available regarding the susceptibility of pregnant goats to artificial infection with *Br. abortus*.

If goats were found to be reasonably susceptible, their use in considerable numbers for experimental work would probably quicken the rate of progress on those phases of the disease where they could be profitably substituted for bovines.

Additional advantages to be derived from their use are the small initial cost, cheapness of maintenance, economy of space, low incidence of sterility and short period of gestation (150 days).

Further, the absence of mastitis greatly facilitates the isolation in pure culture of *Br. abortus* from the milk.

Dose of Br. abortus *used for inoculation.*

In the experiments here recorded infection was induced by instilling into the conjunctival sac a variable number of measured drops of a standard suspension (except in the case of some goats in experiment No. II) of *Br. abortus*.

This suspension consisted of a 0·85 per cent. saline suspension of a 72-hour liver agar growth of a smooth, CO_2-sensitive strain of *Br. abortus* matched in opacity to Brown's No. 10 tube. The No. 10 tube is, according to the table supplied by the makers, the equivalent of 7,684 million *Br. melitensis* organisms per c.c.; no estimate is given for *Br. abortus,* so it has been arbitrarily assumed to be approximately the same as that given for *Br. melitensis.*

In order to avoid repetition this suspension will be referred to as the " standard suspension "; the strains of *Br. abortus* used to make it varied, and particulars of them are given.

Each drop of standard suspension measured approximately 0·05 c.c., representing about 384 million organisms.

Strains of Br. abortus *used.*

Hylan strain isolated 9/1/36 from the milk of a heifer that had aborted.

Poppy strain isolated 6/2/36 from the stomach contents of an aborted fœtus.

Freda strain isolated 13/2/37 from the milk of a heifer that had aborted.

Jones strain isolated 17/4/36 by Dr. G. Slavin from the stomach contents of an aborted fœtus.

Rks strain isolated 19/11/37 from the milk of a cow that had aborted.

Strain "98" isolated from a goat 4/12/36. This was a bovine strain (Poppy) that had been passaged through four goats, extending over a period of 242 days.

These six strains were submitted to the usual tests and proved to be typical, smooth, CO_2-sensitive strains of *Br. abortus.* Tests for pathogenicity on series of guinea-pigs showed five strains to be of average virulence; the sixth strain, "Jones," appeared to have a virulence considerably above the average.

Examination of milk for Br. abortus.

Freshly drawn milk was centrifuged and the cream sown on gentian-violet malachite-green liver-agar plates (Stockmayer, 1933). This medium gave satisfactory results, *Br. abortus* usually being obtained in pure culture.

Milk whey for agglutination tests was obtained by the addition of rennet.

Identification of the Brucella strains isolated.

Each strain of brucella isolated was as a routine measure examined as follows:—

(1) Against a positive *Br. abortus* serum.
(2) For growth aerobically and under 10 per cent. CO_2.
(3) For the production of H_2S.
(4) For growth on Huddleson's dye media.
(5) Thermo-agglutination test for antigenic roughness (Pandit and Wilson, 1932).
(6) Pathogenicity tests with falling dilutions of a suspension (Brown's No. 4 tube) of the organism on a series of 16 guinea-pigs. This was carried out only with the strains isolated in the passage experiments.

Agglutination tests of serum and whey were set up in tubes (5 × 1 cm.) in dilutions of 1:10, 1:25, 1:50, 1:100, 1:200, each tube containing 1 c.c. of antigen. The tests were incubated for 20 hours at 37° C.

TABLE I.

SUSCEPTIBILITY OF PREGNANT GOATS TO ARTIFICIAL INFECTION WITH *Br. abortus*.

EXPERIMENT I.

Number of Goat.	*Strain of* Br. abortus.	*Origin of Strain.*	*Dose Conjunctival Sac.*	*Result.*	*Days after Infecting Dose.*	*Age of Fœtus.*	*Remarks.*
118	"Jones"	Bovine	3 drops	N.P.	108	Full-term	*Br. abortus* isolated from fœtal membranes.
121	"	"	2 "	Abortion	30	87	Fœtal membranes not found. *Br. abortus* isolated from fœtal brain and liver.
122	"	"	1 drop	N.P. Small weak kid	20	Full-term	*Br. abortus* isolated from fœtal membranes and from milk of goat.
104	"	"	3 drops	Abortion	33	60	*Br. abortus* isolated from fœtal membranes and from milk of goat.
119	"	"	2 "	"	69	129	*Br. abortus* cultured from foetal membranes. *Br. abortus* not isolated from milk by culture or guinea-pig inoculation.
128	"	"	2 "	" Two dead fœtuses	72	139	Fœtal membranes not found. Cultures of fœtal stomachs, brains and livers negative. Goat died of metritis. Milk not examined.
117	"Freda"	"	2 "	Abortion	28	84	*Br. abortus* isolated from fœtal membranes and brain and from milk of goat.
47	"	"	2 "	Twins; one dead and undeveloped	49	Full-term	Cultures of brains, livers and stomachs of kids, negative. *Br. abortus* not isolated from milk by culture or guinea-pig inoculation.
126	"	"	3 "	Abortion	35	95	*Br. abortus* cultured from fœtal brain and milk of goat.
124	"	"	3 "	"	56	116	Cultures of fœtal stomach, liver and brain were negative. *Br. abortus* cultured from fœtal membranes and milk of goat.
130	"	"	3 "	N.P.	55	Full-term	Cultures of liver and brain of kid negative. Guinea-pigs inoculated with suspension of fœtal membranes showed no evidence of brucella infection. The milk was not examined as goat died shortly after parturition.

129	„	„	3 „	Abortion	75	124	Cultures of liver and brain of fœtus negative. *Br. abortus* isolated by guinea-pig inoculation from fœtal membranes and milk of goat.
136	„	„	4 „	„	38	74	Membranes not found. Cultures of fœtal liver and brain negative. Guinea-pigs inoculated with milk showed no evidence of brucella infection.
135	„	„	4 „	„	73	106	Membranes not found. *Br. abortus* cultured from milk. Guinea-pigs inoculated with milk showed no evidence of brucella infection.
140	„	„	5 „	„	86	125	Cultures of fœtal brain and liver were negative. *Br. abortus* isolated by guinea-pig inoculation from fœtal membranes and milk of goat.
107	" 98 "	Goat	3 „	N.P.	77	Full-term	Cultures of fœtal liver, stomach and brain negative. Cultures and guinea-pig inoculation of milk negative.
112	„	„	3 „	Abortion	69	120	*Br. abortus* isolated from fœtal membranes and the milk. Cultures of fœtal liver, stomach and brain were negative.
123	„	„	2 „	„	30	83	Fœtal membranes not found. *Br. abortus* isolated from fœtal brain, and stomach and milk of goat.
125	„	„	1 drop	N.P.	116	Full-term	Fœtal membranes not found. *Br. abortus* not isolated from milk either by culture or by guinea-pig inoculation.
115	„	„	2 drops	One dead and two live kids	34	„	Fœtal membranes not found. Cultures of liver, brain and heart blood of dead kid negative. *Br. abortus* cultured from milk, but not isolated by guinea-pig inoculation.
48	„	„	2 „	N.P.	30	„	*Br. abortus* cultured from brain of kid and isolated from milk by guinea-pig inoculation.
99	„	„	6 „	Abortion	25	91	Fœtal membranes not found. *Br. abortus* cultured from fœtal brain and liver and milk of goat.

N.P. = Normal parturition.

The antigen used consisted of washed liver agar growths matched in opacity to Brown's No. 1 tube. The antigen was frequently tested against three preserved sera of different titres.* No significant variation was observed.

Experiment No. 1.

Twenty-two pregnant goats were inoculated *via* the conjunctival sac with different doses of standard suspension of three strains of *Br. abortus* (Table I).

Most of the goats used in this experiment were served at the Institute, but a few were in-kid when bought and their dates of service were unknown.

Goats, 48, 115 and 122, which gave birth to full-term kids, 30, 34 and 20 days respectively after the infecting dose, were too far advanced in pregnancy at the time of inoculation for the results to be of value; *Br. abortus* was, however, isolated from the placenta of 122, from the milk of 115 and from the fœtal brain and the milk of 48.

Five goats (107, 118, 47, 125 and 130) produced full-term kids; *Br. abortus* was isolated from the fœtal membranes of 118.

Fourteen of the remaining 19 goats, *i.e.*, omitting Nos. 48, 115 and 122, aborted (73 per cent.).

The fœtal membranes were not obtained from ten goats—they had presumably been eaten by the goats, but in seven cases *Br. abortus* was recovered from either the fœtal tissues or the milk.

The fœtal membranes were obtained from eight goats that aborted; seven of the membranes were either cultured or inoculated into guinea-pigs and in every instance *Br. abortus* was recovered. The fœtal membranes appeared to be the most constant site of *Br. abortus.*

Experiment No. II.

Fourteen pregnant goats were inoculated *via* the conjunctival sac, seven with standard suspension and seven with a suspension of half that strength (Brown's No. 5 tube) of a bovine strain, Rks, of *Br. abortus.*

In view of the considerable number of goats in Experiment No. I from which the fœtal membranes were not obtained, all goats in experiments II, III, IV were from the thirtieth day after inoculation muzzled during the night and kept under observation during the day.

It will be seen from Table II that five goats carried their kids to term and from one of these (No. 187) *Br. abortus* was isolated from the fœtal membranes and stomach. Nine of the 14 goats aborted (64 per cent.).

Five of the seven goats that received the standard suspension aborted and from six of the seven *Br. abortus* was isolated. Four

* I am indebted to Dr. G. Slavin for making these tests.

TABLE II.

EXPERIMENT II.

GOATS INOCULATED WITH A BOVINE STRAIN ("RKS") OF *Br. abortus*.

Number of Goat.	*Dose Conjunctival Sac.*	*Result.*	*Days after Infecting Dose.*	*Age of Fœtus. Days.*	*Remarks.*
190	1 drop S.	Abortion	36	86	*Br. abortus* cultured from fœtal liver, and isolated by guinea-pig inoculation from a mixed suspension of brain, liver and heart blood.
184	2 drops S.	N.P.	116	Full-term	*Br. abortus* isolated by guinea-pig inoculation from fœtal membranes and milk of goat.
185	2 „	„	114	„	Cultures and guinea-pig inoculation of fœtal membranes and tissues and milk of goat negative.
177	3 „	Abortion	46	93	*Br. abortus* isolated from fœtal membranes and tissues, and from milk of goat.
189	3 „	„	78	123	*Br. abortus* isolated from fœtal membranes and from milk of goat.
137	3 „	„	36	77	*Br. abortus* isolated from fœtal membranes.
176	3 „	„	46	108	*Br. abortus* isolated by guinea-pig inoculation from fœtal membranes and tissues and from milk of goat. *Br. abortus* cultured from fœtal brain.
147	2 drops *	„	88	129	*Br. abortus* isolated by guinea-pig inoculation from fœtal membranes and from milk of goat.
191	2 „	—	115	Full-term	Culture and guinea-pig inoculation of fœtal membranes and tissues and milk of goat negative for *Br. abortus*.
182	2 „	—	106	„	Guinea-pigs inoculated with fœtal membranes and tissues and milk of goat negative for *Br. abortus*.
175	2 „	Abortion	66	118	*Br. abortus* isolated by culture and guinea-pig inoculation from the foetal liver, brain, heart blood and membranes and from milk of goat.
187	3 „	—	97	Full-term	*Br. abortus* cultured from fœtal stomach and isolated by guinea-pig inoculation from fœtal membranes.
179	3 „	Abortion	98	141	*Br. abortus* cultured from fœtal stomach and heart blood and isolated by guinea-pig inoculation from fœtal membranes and from milk of goat.
183	3 „	„	70	117	Fœtal membranes not found. Cultures of fœtal liver, heart blood and brain negative. Guinea-pigs inoculated with fœtal liver and brain negative. *Br. abortus* isolated by guinea-pig inoculation from milk of goat.

S. = Standard suspension. * = Brown's No. 5 tube. N.P. = Normal parturition.

of the seven goats that received the weaker suspension aborted and from five of the seven *Br. abortus* was isolated.

Experiment No. III.

The ten goats in this experiment were in-kid when received at the laboratory and their service dates were unknown.

Table III shows that in four goats (199, 200, 202, 203) pregnancy ended at term, 26, 27, 20 and 26 days respectively after the infecting dose ; *Br. abortus* was isolated from the placenta and milk of three of these goats. In view of the short interval that elapsed between inoculation and parturition in these four animals the results obtained are of little value and need not be further considered.

The remaining six goats aborted (100 per cent.).

Experiment No. IV..

This experiment was carried out late in the breeding season and, of twelve goats believed to be in-kid and inoculated into the conjunctival sac, three subsequently proved to be barren.

The "Freda" strain of *Br. abortus* used for infecting these goats had been preserved in the dried state for one year, and, as judged by the results obtained from the inoculation of a series of 16 guinea-pigs and from the conjunctival infection of goats, no marked change in pathogenicity appeared to have occurred.

It will be seen from Table No. IV that of the nine pregnant goats inoculated two (211 and 217) carried their kids to term and seven aborted (77 per cent.).

Discussion.

Fifty-five pregnant goats were used in the four experiments and of these, 36 aborted and 19 carried their kids to term.

The average interval between infection and abortion for 36 goats was 54 days ; the shortest period was 25 days and the longest 100 days.

Six goats (Nos. 48, 115, 122, 199, 202, 203) that gave birth to full-term kids can reasonably be omitted from consideration as they were too far advanced in pregnancy at the time of inoculation for the results to be of value.

Therefore, of the 48 goats remaining, 12 carried their kids to term and 36 aborted, that is, 75 per cent.

Sufficient evidence has been obtained from these experiments to make it clear that goats are less susceptible than bovines to conjunctival infection with *Br. abortus*; nevertheless their susceptibility is fairly high and, when considered in conjunction with their other advantages, they would appear to be of value as experimental animals for certain types of brucella work.

The Serial Passage of *Br. abortus* through Goats.

The literature contains references to experiments carried out to determine the effect of serial passage through foreign hosts on the cultural and biological characters of brucella strains.

TABLE III.

EXPERIMENT III.

GOATS INOCULATED WITH A BOVINE STRAIN ("FREDA") OF *Br. abortus*.

Number of Goat.	*Dose Conjunctival Sac.*	*Result.*	*Days after Infecting Dose.*	*Age of Fœtus. Days.*	*Remarks.*
186	3 drops	Abortion	34	119	*Br. abortus* isolated by guinea-pig inoculation from fœtal membranes and milk of goat.
196	3 „	„	50	134	*Br. abortus* cultured from fœtal heart blood, and isolated by guinea-pig inoculation from fœtal membranes and milk of goat.
195	3 „	„	45	95	*Br. abortus* cultured from fœtal brain, and isolated by guinea-pig inoculation from fœtal membranes and from milk of goat.
197	3 „	„	73	131	*Br. abortus* cultured from fœtal brain and milk of goat, and isolated by guinea-pig inoculation from fœtal membranes.
204	3 „	„	30	118 (about)	*Br. abortus* isolated through guinea-pigs from fœtal membranes and from milk of goat.
178	3 „	„	74	114	Fœtal membranes not found. *Br. abortus* cultured from fœtal stomach and isolated through guinea-pigs from fœtal liver and brain and from milk of goat.
199	3 „	N.P.	26	Full-term	*Br. abortus* isolated by guinea-pig inoculation from fœtal membranes and milk of goat.
200	3 „	„	27	„	*Br. abortus* isolated by guinea-pig inoculation from fœtal membranes and from milk of goat.
202	3 „	„	20	„	*Br. abortus* not isolated from fœtal tissues or from milk of goat.
203	3 „	„	26	„	*Br. abortus* isolated through guinea-pigs from fœtal membranes and from milk of goat.

N.P. = Normal parturition.

TABLE IV.

EXPERIMENT IV.

GOATS INOCULATED WITH A BOVINE STRAIN ("FREDA" STRAIN) OF *Br. abortus*.

Number of Goat.	*Dose Conjunctival Sac.*	*Result.*	*Days after Infecting Dose.*	*Age of Fœtus. Days.*	*Remarks.*
216	3 drops	Abortion	32	56	Fœtal membranes not found. *Br. abortus* cultured from fœtal brain. Cultures and guinea-pig inoculation of milk negative.
219	3 „	„	29	60	Fœtal membranes not found. *Br. abortus* cultured from fœtal brain and milk of goat.
214	3 „	„	30	70	*Br. abortus* cultured from fœtal liver, brain and heart blood and isolated through guinea-pigs from fœtal membranes and milk of goat.
222	3 „	„	49	70	Fœtal membranes not found. Cultures and guinea-pig inoculation of fœtal brain, liver and stomach contents negative. No milk obtainable.
221	3 „	„	37	56	Fœtal membranes not found. *Br. abortus* cultured from fœtal brain.
212	3 „	„	100	108	*Br. abortus* isolated through guinea-pigs from fœtal membranes. Cultures of fœtal tissues negative. Culture and guinea-pig inoculation of milk negative.
217	3 „	N.P.	130	Full-term	Cultures and guinea-pig inoculation of milk and fœtal membranes negative.
218	3 „	Abortion	96	105	Cultures of fœtal tissues negative. Cultures and guinea-pig inoculation of milk and fœtal membranes negative.
211	3 „	N.P.	114	Full-term	Cultures of fœtal tissues negative. Cultures and guinea-pig inoculation of milk and fœtal membranes negative.

N.P. = Normal parturition.

Weigmann (1931) infected goats subcutaneously and *per os* with an *abortus* strain of human origin. There was no evidence of the strain acquiring the characters of *melitensis*.

Stockmayer (1933) showed that the passage of a bovine strain of *abortus* through pigs brought about no change in its biological characters.

Taylor, Vidal and Roman (1934) found no change in the character of strains of *melitensis* after periods extending from 12-18 months in the udders of naturally infected cows.

Henricsson and Lindström (1936) passaged an *abortus* strain of human origin through seven goats. Infection was induced by feeding suspensions of the organism on three occasions at two days intervals. *Br. abortus* was recovered on the 28th day by blood culture and a second goat was infected with the passage strain in a similar manner to the first. Seven passages were made, and the strain obtained from the seventh goat was tested for pathogenicity on monkeys (*Macacus rhesus*) and submitted to dye and serological tests, but no difference was observed between it and the original strain.

Present Inquiry.

The object of the experiments here recorded was to determine whether the serial passage through goats of a recently isolated strain of *Br. abortus* of bovine origin would:—

(*a*) modify its pathogenicity for bovines;

(*b*) transform it into a caprine strain more closely related to *Br. melitensis*,

and further, to determine the fate of *Br. abortus* when inoculated by different routes into pregnant and non-pregnant goats.

Each goat was inoculated with 10 c.c. standard suspension, that is, roughly 76,840 million bacteria.

Following inoculation, blood tests were made at short intervals and whey tests were made daily. In addition, cream from centrifuged milk was sown on malachite-green gentian-violet liver-agar plates.

When *Br. abortus* had been present for a period in the udder of a goat, a single colony was picked from the dye medium, inoculated on to liver agar and "standard" suspension prepared and inoculated into another goat.

It was intended only to passage the organism when it appeared to be diminishing in numbers in the milk, but some of the goats became emaciated from worm infestation, contracted from grazing on a restricted area, and on this account it was necessary in many cases to shorten the interval between passages.

The "Hylan" and "Poppy" strains of *Br. abortus* were selected for the passage experiments; these strains were never passed through guinea-pigs and in the intervals between goat passages were usually

maintained on culture medium only for the time necessary for routine identification tests.

The test dose given to the in-calf heifers was three drops, each drop being approximately 0·05 c.c. of "standard" suspensions, together representing about 1,152 million bacteria.

This dose was undoubtedly in excess of that required to cause abortion in a high percentage of susceptible bovines, but no information of any value on this point could be found in the literature, and preliminary titration experiments had to be ruled out on the grounds of expense.

The question at issue was whether or not any marked diminution in virulence of the strain for bovines had been brought about by the goat passages; and as only a few in-calf heifers were available for testing this point, it was decided to use a fairly heavy dose. It was realised, however, that with the dose used only a marked change in virulence would be detected.

It was considered advisable in the first instance to confirm the pathogenicity of the two strains by the inoculation of in-calf heifers. Two heifers, Nos. 776 and 777, believed to be in-calf, were inoculated *via* the conjunctival sac with three drops of standard suspension of "Poppy" and "Hylan" strains of *Br. abortus,* respectively. Unfortunately, neither of the animals proved to be in-calf and owing to the considerable difficulty experienced at that time of obtaining in-calf heifers from brucella-free herds this preliminary test was not repeated. In view of the results obtained subsequently in tests on bovines this omission was of little importance.

Experiment No. V.

In this passage series the inoculum for each goat consisted of 10 c.c. standard suspension of *Br. abortus* ("Poppy" strain).

Table V.

Passage.	*Goat Number.*	*Br. abortus. Days in Goat before Passage.*	*Route of Inoculation.*	*Maximum Agglutination Titre.*		*Sub-passages.*
				Blood.	*Whey.*	
1st	39	143	Udder	250	50	From Goat 39
2nd	80	114	„	1,000	25	Heifer 839*
3rd	106	20	I/V	1,000	50	Goat 113
4th	110	8	„	1,000	50	From Goat 80:
5th	133	57	„	1,000	50	Goat 97
6th	134	43	„	2,000	50	Goat 101
Heifer	879*	Total = 385				

* In-calf heifer inoculated *via* conjunctival sac and abortion resulted.

Goat No. 39 (1st passage).

A pregnant goat, not in milk, gave birth to a full-term kid 118 days after the infecting dose. Microscopical examination and guinea-pig inoculation failed to demonstrate *Br. abortus* in the placenta, but the organism was cultivated under CO_2 from the milk ; plates incubated aerobically remained sterile.

The culture isolated on the 143rd day from the milk was used for the inoculation of goat No. 80. The milk examination was discontinued for a time, but on re-examination 257 days after the infecting dose the organism was again isolated under CO_2. A suspension of *Br. abortus* isolated after a sojourn of 259 days in the udder of this goat was inoculated into heifer No. 838. Goat No. 39 died a few days later as the result of an accident.

Heifer No. 838.

In-calf heifer. Inoculated into the conjunctival sac with three drops of standard suspension of *Br. abortus* isolated on the 259th day of infection from the milk of goat No. 39. Fifteen days later the blood titre was 1 : 100, at which it remained until the animal aborted a six-month fœtus 28 days after the infecting dose. A CO_2-sensitive strain of *Br. abortus* was isolated from the stomach contents, liver and bone marrow of the fœtus.

During the following month the blood titre of the heifer gradually rose to 1 : 500 and the whey titre fluctuated between 1 : 50 and 1 : 200. Numerous platings were made of the milk and in addition 14 guinea-pigs were inoculated at intervals with composite samples of it, but *Br. abortus* was not recovered—which was somewhat unusual in view of the high whey titre.

Goat No. 80 (2nd passage).

Pregnant goat. Twenty-five days after inoculation gave birth to a full-term kid. An agglutination test of the kid's blood was negative and cultures of the fœtal membranes remained sterile. *Br. abortus* was present in the milk from the 6th to the 90th day after parturition ; the organism had then been in the udder for 114 days. Nine months later this goat gave birth to two full-term kids, one alive and one dead. Cultural examination of the fœtal membranes and organs of the dead kid failed to demonstrate *Br. abortus* ; and 21 platings of the milk were negative. In addition four guinea-pigs inoculated with composite milk samples showed no evidence of brucella infection. For the results of *post-mortem* examination of this goat see under " Persistence of *Br. abortus* in the tissues."

Goat 106 (3rd passage).

Br. abortus was present in the milk from the 8th to the 40th day, when the examinations were discontinued. The strain isolated from this goat grew aerobically although when originally isolated from goat No. 80 it was CO_2-sensitive. In passaging the organism

it was kept as a rule on culture medium only for the time necessary for identification, usually about a week. In the present instance, however, the strain isolated from goat No. 80 was not re-inoculated for about six weeks into goat No. 106. This is the probable explanation of its acquiring the power of growing aerobically. It was thought at the time that it might be an indication that the character of the strain was changing towards that of *melitensis* as the result of its sojourn in goats, but subsequent experience ruled out this view. A further fact which appeared to support the view that the character of the strain was changing was that *Br. abortus* was obtained by blood culture from this goat three days after parturition.

Goat 110 (*4th passage*).

Br. abortus was present in the milk from the 5th to the 13th day, when examinations were discontinued. The strain isolated grew aerobically, but otherwise its characters were those of *Br. abortus*. As this goat was giving little milk the organism recovered on the 8th day was inoculated into goat No. 133.

Goat 133 (*5th passage*).

An aerobic strain of *Br. abortus* was present in the milk from the 15th to the 71st day, when examinations were discontinued.

Goat 134 (*6th passage*).

An aerobic strain of *Br. abortus* was present in the milk from the 15th to the 90th day, when examinations were discontinued.

Heifer No. 879.

In-calf heifer. Inoculated into the conjunctival sac with three drops of standard suspension of *Br. abortus* isolated on the 43rd day of infection from the milk of goat No. 134. From the 17th to the 34th day the blood titre was between 1 : 200 and 1 : 250.

The heifer aborted a 6-months fœtus on the 45th day after the infecting dose, and an aerobic strain of *Br. abortus* was isolated from the fœtal membranes and from the heifer's milk ; this represented the original "Poppy" strain which had been passaged through six goats, during a period extending over 385 days.

In view of this result the experiment was discontinued as there was no evidence of any marked attenuation in the virulence of the strain for bovines. The original CO_2-sensitive strain had become aerobic and retained this characteristic through subsequent passages, but as already mentioned it is improbable that this change was the result of passage through goats.

Goat No. 97.

Inoculated with a suspension of *Br. abortus* isolated on the 114th day of infection from the milk of goat No. 80. The milk was plated daily from the 9th to the 21st day but *Br. abortus* was not recovered.

The blood titre was at this period 1 : 500 and the whey titre fluctuated between 1 : 10 and 1 : 50.

The goat aborted a mummified fœtus, aged 84 days, on the 46th day after inoculation. There were no changes in the blood and whey titres after abortion had occurred, but the organism was now readily isolated from the milk.

For the results of *post-mortem* examination see under " Persistence of *Br. abortus* in the Tissues."

Goat No. 101.

Inoculated into the teat canal with suspension of *Br. abortus* isolated on the 114th day of infection from the milk of goat No. 80.

Thirty-nine days later the goat aborted a nearly full-term dead fœtus, and *Br. abortus* was isolated from the fœtal liver and stomach. An agglutination test of the fœtal blood was negative. *Br. abortus* was isolated from the milk the day after abortion and was execreted throughout the following month, at which point the examinations were discontinued.

The strain of *Br. abortus* isolated from the milk grew aerobically, although the culture used for inoculation was at the time of isolation CO_2-sensitive. This is the same strain as was used for the inoculation of goat No. 106 ; it was maintained on artificial media for 30 days between its isolation from goat No. 80 and its inoculation into goat No. 101.

Seven months later, when the blood titre was 1 : 100 and the whey titre 1 : 10, the goat gave birth to two full-term healthy kids. Cultural examination of the milk was negative ; samples were inoculated into eight guinea-pigs, but these showed no evidence of brucella infection.

Experiment No. VI.

In this passage series 10 c.c. " standard " suspension of *Br. abortus* (Hylan strain) was inoculated intravenously into all goats except No. 102.

Goat No. 43 (*1st passage*).

The organism was recovered from the milk two days after inoculation when the blood and whey were still negative to the agglutination test in a 1 : 10 dilution.

As this goat was weak and emaciated as the result of a heavy worm infestation, the strain of *abortus* isolated on the sixth day from the milk was inoculated into the next goat of the series, and goat No. 43 was destroyed.

Br. abortus was first isolated from the milk on the 15th day and was excreted continuously for at least 70 days, after which the examinations were temporarily discontinued. Cultural examination of the milk was made almost daily from the 180th to the 210th

TABLE VI.

Passage.	*Goat Number.*	*Br. abortus. Days in Goat before Passage.*	*Maximum Agglutination Titre.*		*Sub-passages.*
			Blood.	*Whey.*	
1st	43	6	200	50	
2nd	49	34	500	25	
3rd	66	12	2,500	25	From Goat 66 :
4th	94	13	2,000	50	Goat 62
5th	100	255	500	50	From Goat 62 :
		Total = 318			Heifer 818*
	Heifer 877*				Goat 92
	Heifer 881*				Goat 67
					From Goat 94 :
					Goat 102

* In-calf heifer inoculated *via* conjunctival sac and abortion resulted.

day, but the organism was not again isolated, although the whey titre was about 1/100 and the blood titre about 1 : 500.

Four guinea-pigs inoculated with milk drawn on the 180th day became infected, and *Br. abortus* was isolated from them.

Goat No. 66 (*3rd passage*).

Br. abortus was present in the milk from the 9th to the 21st day, when examinations were temporarily discontinued. Cultural examinations of milk daily from the 184th to the 196th day were negative, and four guinea-pigs inoculated with milk taken on the 184th day showed no evidence of brucella infection.

This goat gave birth to two full term kids 256 days after the infecting dose. Cultural examination and guinea-pig inoculation of milk, fœtal membranes and tissues (liver, spleen, stomach-contents and brain) of the kids gave negative results.

Goat No. 62 (*4th passage*).

The organism was cultured from the milk from the 3rd to the 60th day, throughout which period the whey was negative to the agglutination test.

Heifer No. 818.

In-calf heifer. Inoculated into the conjunctival sac with three drops of standard suspension of *Br. abortus,* isolated on the 30th day of infection from the milk of goat No. 62.

This strain had been passaged four times through goats over a period of 80 days. The blood titre was 1 : 10 on the 9th day, 1 : 50 on the 17th day and 1 : 100 on the 24th day.

The heifer aborted an eight-months fœtus on the 35th day after inoculation. *Br. abortus* was isolated from the fœtal membranes and fœtal stomach. Frequent cultural examination of the milk for a period of one month failed to isolate *Br. abortus*. Six guinea-pigs inoculated with a pooled sample of milk taken the day after abortion, and four guinea-pigs inoculated with a pooled sample taken on the 16th day, showed no evidence of brucella infection.

During the first and second days after abortion the whey titre was 1 : 50, but it fell rapidly and by the sixth day was negative in a dilution of 1 : 10. The transient appearance of agglutinins in the milk immediately after abortion was apparently the result of an overflow from the blood and not of a local production in the udder.

Goat No. 94 (*4th passage*).

Br. abortus was recovered from the milk on the 13th day. The goat was then slaughtered on account of emaciation as the result of worm infestation.

Goat No. 100 (*5th passage*).

The organism was recovered from the milk on the 4th day and was excreted continuously up to the 255th day, when the examinations were discontinued. The goat gave birth to two full-term weakly kids 162 days after the infecting dose, and *Br. abortus* was isolated from the fœtal membranes. The strain obtained from the milk on the 255th day of infection was mainly CO_2-sensitive, but one or two colonies developed on plates incubated aerobically. This strain was used for the inoculation of two in-calf heifers, Nos. 877 and 881. *Br. abortus* was still present in the udder of this goat when autopsied 386 days after infection and had apparently become permanently established there.

For *post-mortem* results see under "Persistence of *Br. abortus* in the Tissues."

Goat No. 92.

The blood titre was 1 : 100 on the 8th day and 1 : 250 on the 11th and 17th days. The whey titre was 1 : 50 on the 6th day, 1 : 200 on the 10th day, and negative in 1 : 10 on the 21st day. The milk was plated daily from the 4th to the 25th day, but *Br. abortus* was not isolated and two groups of four guinea-pigs inoculated with milk obtained on the 10th and 25th days respectively showed no evidence of brucella infection.

Goat No. 67.

The blood titre was 1 : 5,000 on the 13th day, 1 : 500 on the 28th day and 1 : 100 on the 44th day. The whey titre was 1 : 25 on the 5th day, 1 : 100 on the 8th day and negative in 1 : 10 by the 30th day.

The milk was plated daily from the 5th to the 43rd day, but *Br. abortus* was not recovered. Four guinea-pigs inoculated with milk obtained on the 51st day showed no evidence of brucella infection.

For the results of *post-mortem* examination see under "Persistence of *Br. abortus* in the Tissues."

In view of the heavy inoculum used, it is surprising that *Br. abortus* was not recovered from the udders of goats Nos. 67 and 92. Part of the suspension with which they were inoculated caused abortion in heifer No. 818. Both goats were negative on two occasions to the agglutination test before being inoculated. They were apparently strongly resistant to infection and this may possibly have been the result of an acquired immunity from contact with brucella infected cattle before arrival at the laboratory.

Heifer No. 877.

In-calf heifer. Inoculated into the conjunctival sac with three drops of standard suspension of *Br. abortus,* isolated on the 255th day of infection from the milk of goat No. 100. This represented the original "Hylan" strain which had been passaged through five goats over a period of 318 days. On the 36th day the blood titre was 1:100 and on the 54th day 1:500. The heifer aborted a seven-months' fœtus on the 63rd day after the infecting dose; two days later the blood titre was 1:1,000. A CO_2-sensitive strain of *Br. abortus* was isolated by guinea-pig inoculation from the fœtal membranes and from the heifer's milk.

Heifer No. 881.

In-calf heifer. Inoculated *via* the conjunctival sac with three drops of the same suspension as that used for the inoculation of heifer 877. On the 11th day the blood titre was 1:250, and on the 28th day 1:200. The heifer aborted a six-months' fœtus on the 86th day after the infecting dose; the day after abortion the blood titre was 1:500. A CO_2-sensitive strain of *Br. abortus* was isolated by guinea-pig inoculation from the fœtal membranes and from the heifer's milk.

Summary.

Two strains of *Br. abortus,* of bovine origin, were passaged through series of goats.

One strain, "Poppy" (Experiment No. V), was passaged through six goats; two goats were inoculated into the udder and four intravenously.

After a period of 259 days in the udder of a goat the strain was tested for pathogenicity by conjunctival infection of an in-calf heifer. The heifer aborted. The strain was again tested on an in-calf heifer after passage through six goats, extending over a period of 385 days; the heifer aborted.

The other strain, "Hylan" (Experiment No. VI) was passaged intravenously through five goats. This strain, after passage through

four goats, extending over a period of 80 days, was tested on an in-calf heifer; the heifer aborted. The strain was again tested on an in-calf heifer after passage through five goats, extending over a period of 318 days, and the heifer aborted.

The strain isolated from each goat, in both experiments, was tested for pathogenicity by inoculation in falling dilutions of a standard suspension into a series of 16 guinea-pigs. There was no evidence of any change in pathogenicity of either strain for guinea-pigs as the result of goat passages. Tests for H_2S and for dye sensitivity showed no differences between the original bovine strains and those obtained after passage through goats.

There is a limited amount of data in the literature regarding the susceptibility of pregnant bovines to inoculation into the conjunctival sac with *Br. abortus*. Schroeder and Cotton (1924) induced abortion in a heifer by instilling one drop of an *abortus* suspension into the conjunctival sac. Cotton and Buck (1931) reported that 16 out of 17 pregnant heifers aborted following inoculation into the conjunctival sac of two to four drops of an *abortus* suspension approximately 20 times the density of tube No. 1 of McFarland's nephelometer.

During the course of this investigation five pregnant heifers were inoculated into the conjunctival sac with *abortus* suspensions and all aborted. It would appear, therefore, that pregnant bovines are highly susceptible to conjunctival infection with *Br. abortus*.

Pathogenicity Tests on Guinea-Pigs of *Br. abortus* Strains Before and After Passage through Goats.

It was recognised before starting these tests that the use of guinea-pigs is of relatively little value for the detection of fine differences in pathogenicity between strains of *Br. abortus*. It was, however, considered that they would be of some value for the detection of gross changes in pathogenicity, should such changes occur as the result of serial passage of the organism through goats. The number of large animals that were available for testing this point were necessarily limited on the grounds of expense.

The "Poppy" and "Hylan" strains of *Br. abortus*, used in passage series Nos. I and II respectively, were each tested for pathogenicity in falling dilutions of a standard suspension (Brown's No. 4 tube) on a series of 16 guinea-pigs, both before inoculation into goats and on their isolation from individual goats. The organism was suspended in 0·85 per cent. salt solution and the suspension matched in opacity to Brown's No. 4 tube, which is, according to the table supplied with the tubes, the equivalent in opacity of a suspension of 3,074 million *Br. melitensis* per c.c.; it has been arbitrarily assumed that a similar suspension of *Br. abortus* contains approximately the same number of organisms. From the standard suspension falling dilutions were made of 10^{-2}, 10^{-3}, 10^{-4}, 5×10^{-4}, 10^{-5}, 10^{-6}, 2×10^{-6}.

A separate pipette was used for each dilution. Two guinea-pigs were inoculated intraperitoneally with 1 c.c. of each dilution.

The guinea-pigs were killed on the 44th day, agglutination tests made of the blood, the spleen cultured on to liver agar and according to its size and appearance the results indicated as + + + +, + + +, + +, + and −.

It is generally recognised that absence of change in the size or appearance of the spleen or absence of specific agglutinins in the blood of guinea-pigs is not absolute evidence of freedom from brucella infection.

Br. abortus was isolated from the 26 goats used in the passage experiments and the culture obtained from each goat was tested for pathogenicity as described on a series of guinea-pigs.

The highest dilution (2×10^{-6}) containing approximately 1,537 bacteria, was inoculated into 52 guinea-pigs, 49 of which were subsequently autopsied, 32 yielded cultures of *Br. abortus* and 17 were sterile. Hagan (1922) computed that somewhat less than 100 *Br. abortus* organisms were sufficient to infect most guinea-pigs. It should be noted, however, that his strain was of guinea-pig origin, whereas the strains used here were of goat origin. Scorgie (1938) found that 28 to 280 *Br. abortus* organisms caused infection in six out of nine guinea-pigs.

It would serve no useful purpose here to give details of the results obtained with each group of guinea-pigs, as not only were there variations between some groups, but there were at times discrepancies between different dilutions within individual groups. Nevertheless, when the results were viewed as a whole, there was no evidence of change in pathogenicity for guinea-pigs of either strain of *Br. abortus* as the result of serial passage through goats.

Excretion of *Br. abortus* in Milk.

Observations by Meyer and Eddie (1935) and Taylor and his co-workers (1938) suggest that the majority of goats naturally infected with *melitensis* cease eventually to eliminate the organism in the milk.

The experiments here recorded do not permit of an accurate estimate of the duration of excretion of *Br. abortus* in the milk of individual goats. The milk of each goat was plated daily for variable periods, but as soon as the strain was transferred to a new goat, the daily examinations were discontinued and samples tested only occasionally to ascertain if *Br. abortus* was still being excreted. It soon became evident from the daily examinations that *Br. abortus* is excreted intermittently in goat's milk, as it is in cow's milk. Many goats used in the passage experiments were kept under observation for only two to three months and in a majority of them the organism was still present in the udder at the time of slaughter.

Br. abortus was not recovered, either by culture or by passage through guinea-pigs, from the milk of two goats (Nos. 67 and 92), in

spite of the heavy inoculum used. *Br. abortus* was isolated either culturally or through guinea-pigs from the milk of about 60 per cent. of goats inoculated into the conjunctival sac. The presence or absence of *Br. abortus in* goats' milk would appear to depend to some extent on the period of gestation at which abortion occurs, the later the stage of gestation the greater the probability of its being present.

The organism persisted for a considerable period in the udders of some goats, *e.g.*, 259 days in goat No. 39, 114 days in goat No. 80, 386 days in goat No. 100, and 216 days in goat No. 49 ; these figures refer only to the last examination made. It is possible that the organism may have become permanently established in the udders of some of these animals.

The Persistence of *Br. abortus* in the Tissues.

The principal lymphatic glands and organs of eleven goats, autopsied at varying intervals after infection, were cultured and in addition saline suspensions of them were inoculated (2 c.c.) into 44 guinea-pigs ; half of each group were inoculated intraperitoneally and half intramuscularly. The following tissues were examined: the popliteal, precrual, prescapular, pharyngeal, submaxillary, bronchial, mediastinal, portal, iliac, mesenteric, and supramammary lymphatic glands and the liver, spleen, kidney, udder, uterine scrapings, blood and milk.

Table VII shows the results of the examinations.

These results suggest that the majority of goats are strongly resistant to permanent infection with *Br. abortus,* but that inoculation may occasionally give rise to a more or less permanent infection.

Natural Infection of Goats with *Br. abortus.*

There appear to be few records in the literature on the subject of natural infection of goats with *Br. abortus.*

Haddow (1934) in India carried out agglutination tests of two large herds of goats and found a considerable number of reactors. An organism was isolated which was stated to be more closely related serologically to *Br. abortus* than to *Br. melitensis.*

Meurou and Pineau (1937) reported a case of abortion in a goat that had been housed with an aborting heifer. *Br. abortus* was isolated from the milk and blood of the goat. An examination was made of the heifer's milk, apparently for the first time, three months after abortion and *Br. abortus* was isolated. The heifer had been inoculated eleven months previously with live abortion vaccine.

A second goat showed a positive agglutination reaction and gave birth to three normal kids. The fœtal membranes and milk were apparently not examined for *Br. abortus.*

Holth (1937) in Norway reported that on a farm where contagious abortion was prevalent amongst the cattle, 14 out of 17 goats gave positive reactions to the agglutination test.

TABLE VII.

SUMMARY OF *Post-mortem* EXAMINATIONS OF GOATS.

Number of Goat.	*Method of Infection.*	*Normal Parturition or Abortion.*	*At Time of Autopsy.*		*Autopsy. Days after Infecting Dose.*	*Cultural and Biological Examination at Autopsy. Br. abortus.*	*Remarks.*
			Blood Titre.	*Whey Titre.*			
67	I/v	Non-pregnant	200	—	75	Not isolated	*Br. abortus* was never recovered from this goat.
100	I/v.	Normal parturition	100	10	386	Milk	*Br. abortus* was excreted in the milk throughout the period of observation.
97	I/v.	Abortion	250	Partial 10	182	Iliac lymphatic gland	*Br. abortus* was present in the milk when examined on the 60th day after inoculation but later examinations failed to isolate it.
134	I/v.	Non-pregnant	250	—	241	Kidney	*Br. abortus* isolated from milk on the 43rd day after inoculation but later examinations failed to isolate it.
80	Udder	Normal parturition	100	—	341	Not isolated	*Br. abortus* isolated from the milk on the 114th day after inoculation, but later examinations failed to isolate it.
102	„	Abortion	100	—	113	Submaxillary and mediastinal lymphatic glands	*Br. abortus* was present in the milk on the 95th day after inoculation, but later examinations failed to isolate it.
113	„	„	100	—	240	Not isolated	*Br. abortus* was present in the milk on the 90th day after inoculation, but later examinations failed to isolate it.
73	„	Normal parturition	250	—	221	„	This goat was not in-milk at time of inoculation. First milk examinations were made after parturition, 149 days after inoculation, when numerous attempts to isolate *Br. abortus* failed.
107	Conjunctival sac	Dead full-term kid	50	—	154	„	*Br. abortus* was never recovered from this goat.
47	„	2 dead kids; 1 undeveloped	25	—	128	Bronchial lymphatic glands	*Br. abortus* was never isolated from the milk.
119	„	Abortion	100	—	249	Not isolated	*Br. abortus* cultured from the placenta, but not from the fœtal tissues or from the tissues of the goat.

It is of interest to note here that Cotton and Buck (1938) have shown that cattle possess considerable resistance to natural infection with *Br. suis* and seldom abort as the result of direct contact with infected pigs.

In order to obtain some further information on this phase of the disease, two experiments were carried out. Six pregnant goats (Nos. 111, 131, 193, 180, 181, 198) negative to the agglutination test, were put in a pen measuring 18 ft. by 12 ft. Fourteen *Br. abortus* infected goats, immediately after or within a few hours of aborting, were introduced into the pen over a period of 55 days; two goats were put in on the 1st day, two on the 10th, and one each on the 20th, 25th, 32nd, 40th, 41st, 42nd, 44th, 48th, 53rd and 55th days respectively. These goats had been used in other experiments and had been inoculated into the conjunctival sac with *Br. abortus,* nine with "Rks" strain and five with "Freda" strain. When placed in the pen most of them had a vaginal discharge, and many had not yet passed the fœtal membranes. Meyer and Eddie (1935) state that *Br. melitensis* may persist for as long as 41 days in the vaginal discharge of goats. Taylor, Lisbonne, Vidal and Hazemann (1938) found that *Br. melitensis* was present for about 26 days in the vaginal discharge of sheep after abortion. There does not appear to be any information available regarding the length of time *Br. abortus* may persist in the vaginal discharges of goats after abortion, but assuming it to be about the same period as in cows, there was ample opportunity in this experiment for the in-contact pregnant goats to contract infection.

Blood tests of the pregnant goats were made at weekly intervals throughout the experiment. All tests were negative until after parturition, when three goats showed reactions and from two of these *Br. abortus* was isolated from the fœtal membranes by guinea-pig inoculation.

TABLE VIII.

SHOWING THE RESLT OF CONTACT BETWEEN PREGNANT GOATS AND GOATS RECENTLY ABORTED.

Pregnant Goat. Number.	*Days in Pen before Parturition.*	*Parturition.*	*Agglutination Titre of Blood after Parturition.*	*Cultural and Guinea-pig Inoculation of*	
				Milk.	*Fœtal Membranes.*
111	81	Normal	1 : 10	Negative	*Br. abortus*
131	81	„	—	„	Negative
193	85	„	1 : 50	„	*Br. abortus*
180	94	„	—	„	Negative
181	100	„	1 : 10	„	„
198	101	„	—	„	„

Although a heavy infection with *Br. abortus* must have existed in the pen all the pregnant goats carried their kids to term. As will be seen from Table VIII *Br. abortus* was isolated from the fœtal membranes of two of them.

A second experiment was carried out on the same lines. Three pregnant goats (Nos. 105, 108 and 111) were placed in a pen with three goats that had aborted a few hours previously. Seven more goats, immediately after aborting, were added to the pen during the following three weeks. The three pregnant goats carried their kids to term and *Br. abortus* was not isolated from either the fœtal membranes or from the milk (see Table IX).

TABLE IX.

SHOWING THE RESULT OF CONTACT BETWEEN PREGNANT GOATS AND GOATS RECENTLY ABORTED.

Pregnant Goat Number.	*Days in Pen before Parturition.*	*Parturition.*	*Agglutination Titre of Blood after Parturition.*	*Cultural and Guinea-pig Inoculation of*	
				Milk.	*Fœtal Membranes.*
105	72	Normal	Negative	—	—
111	70	"	—	—	—
108	69	"	—	—	—

The results obtained in these experiments suggest that pregnant goats are strongly resistant to natural infection with *Br. abortus.*

BLOOD CULTURES.

There are considerable differences in the results reported in the literature on blood cultures of goats artificially infected with *Br. abortus.*

Burnet (1928) made 30 blood cultures from nine goats infected with abortus and obtained only one positive result, whereas 35 blood cultures from 15 goats artificially infected with *melitensis* gave 23 positive results.

Van der Hoeden (1933) infected an adult he-goat by instilling into the conjunctival sac ten drops of an *abortus* suspension. The organism was recovered by blood culture on the 3rd, 4th and 11th days after inoculation.

Henricsson and Lindström (1936) passaged an *abortus* strain of human origin through seven goats, recovering it by blood culture from each goat. The origin of the strain used in this instance may possibly have had some influence on the facility with which it was isolated from the blood.

During the experiments here reported, 63 blood cultures were made from 40 artificially infected goats; these goats represented infection with three bovine strains, and one bovine goat-passage strain of *Br. abortus.* In the early stage of the work 2 c.c. of blood was drawn from the jugular vein, inoculated into liver broth, incubated under CO_2 and subsequently plated at three-day intervals on to liver agar. This technique was later superseded by that recommended by Gould and Huddleson (1937). This consists in adding

10 c.c. of blood to 10 c.c. of sterile 4 per cent. sodium citrate in saline solution and incubating for 20 days under CO_2. At four-day intervals 0·5 c.c. of blood citrate mixture is inoculated on a liver agar slant.

The blood cultures were made either one or two days after abortion had occurred or within a week of artificial infection. Positive blood cultures were obtained from three goats; from No. 137 immediately after abortion, from No. 104 on the second day after abortion and from No. 106, a non-pregnant goat, on the seventh day after an intravenous inoculation (10 c.c.) of "standard" suspension. In addition to the cultural examinations, blood samples from seven goats that had recently aborted were inoculated intramuscularly (1 c.c.) into each of two guinea-pigs. No evidence of brucella infection was obtained.

Further evidence in support of these results was obtained during the bacteriological examination of the tissues of ten artificially infected goats, killed at variable periods infection, when a blood sample from each animal was inoculated intramuscularly (1 c.c.) into each of two guinea-pigs. The results were negative.

This is in contrast to *melitensis* infection of goats in which the organisms are present in the blood for a variable time after infection.

Meyer and Eddie (1935) found that in *melitensis* infection of goats, a bacteraemia may persist for 59 days or longer.

With regard to the condition in man Topley and Wilson (1936) state:

"Though blood culture is positive in a high proportion of *melitensis* and *suis* infections, not more than 10-20 per cent. of *abortus* infections prove positive by this method."

Our results support those reported by Burnet and suggest that after artificial infection of goats with true bovine strains of *Br. abortus*, or within a short period of abortion occurring, a transient bacteraemia occasionally occurs.

Experimental Goats.

The histories of the goats used in these experiments were unknown, as they were purchased through dealers in different parts of the country, the majority coming from Wales.

Out of a total of about 200 goats tested three showed low blood titres (1 : 10) on arrival at the laboratory. One goat (No. 204) ceased to react within one month. An agglutination test of the whey was negative, while cultural and biological examinations of the milk showed no evidence of brucella infection. The other two reacting goats (312 and 324) are still under observation; in one the titre has fallen and now shows only a slight reaction at 1 : 10; in the other a complete 1 : 10 titre still persists.

The two goats (Nos. 67 and 92) that proved refractory may have been at some time in contact with brucella infected cattle and acquired immunity. Neither of these animals showed any reaction in agglutination tests of the blood and whey before inoculation.

This considerable experience would suggest that it is reasonably safe in this country to use goats, bought in the open market, for brucella experimental work.

Conclusions.

Forty-eight pregnant goats were tested for susceptibility by conjunctival infection with measured doses of *Br. abortus*. Twelve goats carried their kids to term and 36 aborted (75 per cent.).

Sixty-three blood cultures were made from 40 goats artificially infected with *Br. abortus* and three positive results were obtained. This finding is in marked contrast with the high percentage of positive blood cultures obtained from goats infected with *Br. melitensis*.

Two experiments were carried out in which pregnant goats were placed in contact with goats recently aborted. The pregnant goats kidded normally, but three showed agglutination titres after kidding and *Br. abortus* was isolated from the fœtal membranes of two of them.

The tissues of eleven artificially infected goats were examined at varying periods after infection for the presence of *Br. abortus*. The organism was recovered from five animals 113, 128, 182, 241 and 386 days respectively after infection.

Br. abortus was still present in the milk of four goats 114, 216, 259 and 386 days respectively after infection.

Two strains of *Br. abortus*, of bovine origin, were subjected to serial passage in goats and tested subsequently for pathogenicity on in-calf heifers and guinea-pigs. There was no marked change in the pathogenicity of the strains as the result of passage through goats.

The two original strains, "Hylan" and "Poppy," and the passage strains isolated from the goats were examined simultaneously for the production of H_2S and for dye sensitivity, but there was no appreciable difference in the results obtained.

References.

Burnet, E. (1928). *Arch. Inst. Pasteur, Tunis*, **17**, 108-127.
Cotton, W. E., and Buck, J. M. (1931). *J. Amer. vet. med. Ass.*, **78**, 306-325.
——— & ——— (1938). Tech. Bull. U.S. Dep. Agric., No. 269.
Giles, M., Peres, G., and Culty. (1932). *Rev. gen. med. vet.*, **41**, 476-481.
Gould, S. E., and Huddleson, I. F. (1937). *J. Amer. med. Ass.*, **109**, 1971-74.
Haddow, J. R. (1934). Rep. Imp. Inst. Vet. Res., Muktesar, India.
Hagan, W. A. (1922). *J. exp. med.*, **36**, 697-709.
Henricsson, E., and Lindström, B. (1936). *Zlb. Bakt. I. (Orig.)*, **136**, 445-451.
Holth, H. (1937). *Norsk vet. Tidsskr.*, **49**, 524-536.
Meurou, P., and Pineau, M. (1937). *Bull. Acad. vet. France*, **10**, 90-93.
Meyer, K. F., and Eddie, B. (1935). *J. Amer. vet. med. Ass.*, **86**, 286-303.
Pandit, S. R., and Wilson, G. S. (1932). *J. Hyg., Camb.*, **32**, 45-54.

Parisot, J., Vidal, L. F., and Levy, R. (1932). *C.R. Soc. Biol. Paris*, **110,** 78-79.
Schroeder, E. C., and Cotton, W. E. (1924). *J. Amer. vet. med. Ass.*, **66,** 550-561.
Scorgie, N. J. (1938). *J. Path. Bact.*, **46,** 165-171.
Stockmayer, W. (1933). *Berl. tierärztl. Wschr.*, **49,** 741-744.
——— (1933). *Zbl. Bakt. I. (Orig.)*, **128,** 205-216.
Taylor, R. M., Lisbonne, M., and Roman, G. (1932). *Rapp. 1er Congr. Internat. Hyg., Mediterraneenne*, **1,** 706-713.
——— Vidal, L. F., and Roman, G. (1934). *C.R. Soc. Biol. Paris*, **116,** 132-134.
——— Lisbonne, M., Vidal, L., and Hazemann, R. H. (1938). *Rev. med. vet., Toulouse*, **90,** 185-205.
Topley, W. W., and Wilson, G. S. (1936). The Principles of Bacteriology and Immunity. London: Edward Arnold. (2nd Edit.) p. 1343.
Van der Hoeden, J. (1933). *J. comp. Path.*, **46,** 232-247.
Weigmann, F. (1931). *Zbl. Bakt. I. (Orig.)*, **121,** 318-328.
Zwick, and Zellar, H. (1913). *Arb. Gesundh. Amt.*, **43,** 1-128.

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AN ESTIMATE OF A SUITABLE INFECTIVE DOSE OF *BR. ABORTUS* FOR IMMUNISATION TESTS ON CATTLE*

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The experiments recorded in this paper were made in an attempt to determine a suitable infective dose of *Br. abortus* that might be used to test the immunity of pregnant cattle when the bacteria were applied to the surface of the eye. Studies in this laboratory had shown that *Br. abortus* could be preserved *in vacuo* over P_2O_5 for long periods of time without loss of virulence or CO_2 sensitivity; consequently if the average infective dose of a suitable preserved strain was known this dose could be used as required on a future occasion and would be of value in testing the immunity of vaccinated animals. In the majority of vaccination studies on cattle the vaccinated and control animals have been exposed to infection by a "natural" route and not by parenteral inoculation, and in the present experiment this precedent was followed. The eye route was selected because it appeared to offer a more accurate way of bringing relatively small numbers of bacteria into intimate contact with body surfaces through which they might penetrate, than would have been obtained by feeding the organisms or applying them to the mucous surface of the vagina or some other structure, or by bringing them into contact with either the scarified or normal skin.

It is surprising that a small infective dose for cattle has not been estimated before, but apart from considerations of expense two plausible explanations present themselves. In the first place cattle frequently react irregularly to experimental infection when the criterion of infection is the demonstration of *Br. abortus* in either the fœtus or fœtal membranes, or in the colostrum; some animals may remain uninfected although the infective dose may have been very large and the bacteria may even have been inoculated into the body. This variability of cattle may have been regarded as an indication that the size of the infective dose was of little moment, especially when the animals had been exposed to infection by one or other of the "natural" routes. Secondly, the good results that have been obtained when animals vaccinated with living virulent strains of *Br. abortus* and control animals have been exposed to heavy experimental infections, have probably obscured the need for less

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drastic and more accurate methods of testing immunity. These results have been justly regarded as evidence of immunity to *Br. abortus,* but when the magnitude of the infective doses so often used is considered the vaccinated animals frequently showed a remarkably efficient immunity. At the present time it appears unlikely that immunisation by means of dead vaccine or vaccines that are incapable of setting up an infection will produce such a satisfactory immunity as vaccination with living virulent culture. Nevertheless there is evidence that encourages the belief that non-virulent living vaccines will confer some protection which is demonstrable when the infective dose is not too large. Our work on guinea-pigs, some of which has been published (McEwen and Roberts, 1936, McEwen and Priestley, 1938), has shown that vaccination of these animals with living cultures of reduced virulence produced a definite immunity that is only demonstrable when the infective dose is not too large, and it is likely that the same principle will apply to cattle and other animals.

A number of references to immunisation and infection experiments on cattle are quoted to illustrate the points mentioned. Birch and Gilman (1925) fed each of nine cattle when in their third month of pregnancy, with 10 c.c. of a suspension of *Br. abortus* equivalent to 0·75 cm. on the Gates' scale and repeated the treatment three times weekly for a period of three months. Six of the animals aborted and *Br. abortus* was recovered from the fœtuses or membranes of seven of them. Three of the animals produced strong calves. This experiment illustrates the irregularity with which uterine infection and abortion may occur in animals exposed to a heavy and repeated oral infection although it should be noted that all the animals gave a positive reaction to the agglutination test after exposure and *Br. abortus* was recovered from all but one of them. Many other comparable results may be referred to; see for instance, M'Fadyean and Stockman (1909), M'Fadyean, Sheather and Minett (1913), Birch and Gilman (1922, 1926) and Rettger, McAlpine, White and Johnson (1926).

In a vaccination experiment Buck and Creech (1924) exposed eleven pregnant cattle vaccinated with a living culture of *Br. abortus* and eight pregnant controls to infection by feeding 5 to 60 c.c. of the stomach contents of aborted fœtuses to each, some of the animals being fed more than once. Nine of the vaccinated animals calved normally and were free from uterine infection but one aborted and another gave birth to a premature calf and both of these animals had a uterine infection. Seven of the controls aborted and all were infected with *Br. abortus.*

Hart and Traum (1925) infected a group of 17 pregnant heifers vaccinated with a virulent strain of *Br. abortus* and ten control pregnant heifers by feeding a mixture of 8 gallons of infected milk, one gallon of fœtal tissues (lungs, stomach contents and intestinal contents from six fœtuses) emulsified in saline and 4 gallons of dilute broth culture (200 c.c. or more to one gallon) and washings from agar cultures. Each of the animals was drenched with one pint of this heterogeneous mass of infective material and fed hay soaked in it. Fourteen of the vaccinated animals gave birth to normal calves; of the remainder one was accidentally killed, one aborted and another gave birth to a weak calf that died 72 hours later, but none had a uterine infection. On the other hand, six of the controls aborted and one gave birth to a weak calf that had infected membranes. Three calved normally and had no

uterine infection but one of these calved too soon after the infective material had been given for infection of the uterus to have occurred.

These two immunisation experiments were carried out with living virulent vaccines and they show that a high degree of immunity had been produced. It will now be of interest to refer to some results of immunisation experiments where vaccines of modified virulence were used.

Cotton (1932) reported on an experiment where small groups of cattle were vaccinated with three different strains of Brucella. One of these was a porcine strain and need not be considered here. The other two strains are of interest as one was virulent while the other was of reduced virulence and did not produce macroscopic lesions in guinea-pigs two months after inoculation into the animals. The cattle were inoculated with vaccine before conception and when pregnant both vaccinated and control groups were exposed to infection by feeding them on several occasions with diseased organs from guinea-pigs, with fœtal and placental material from aborting cows and with cultures. In addition, on two occasions infective material was dropped on to the eye. Although it is impossible to assess the number of organisms given, the exposure was probably severe. There were five animals in the group vaccinated with the culture of reduced virulence ; four in the group given virulent vaccine and seven controls. Three of those receiving vaccine of reduced virulence aborted and had infected udders, while one of the remaining two that calved normally had a uterine infection. None of those given the virulent vaccine aborted but two had infected udders. Five out of the seven controls aborted and four showed infection of the udder. This experiment suggests that the virulent vaccine conferred the greater degree of resistance.

In the same paper Cotton refers to a small experiment which was carried out to test the effect of vaccinating pregnant animals with the vaccine of reduced virulence. There were four vaccinated and six control animals and the exposure to infection was comparable to that used in the previous experiment. One of the vaccinated animals aborted and two gave birth to full-term calves that had infected membranes. Only one of the vaccinated animals had an infected udder. Three of the controls aborted and were also infected in the udder, three calved normally but one showed a uterine infection. These results do not reflect favourably on the vaccine of reduced virulence. However, a less severe exposure to infection might have given more favourable results.

Cotton and Buck (1933) carried out a further experiment on the vaccination of pregnant cattle with a strain of very reduced virulence. This strain did not produce lesions in guinea-pigs inoculated subcutaneously or intraperitoneally with relatively large doses ; but it was recovered from the spleens of two of six guinea-pigs killed six weeks after inoculation, but not from twelve guinea-pigs killed two months after inoculation. A culture of the strain was inoculated into one pregnant heifer but the animal calved normally and was not affected. Milk from three animals inoculated with the strain during pregnancy was collected on several occasions and inoculated into guinea-pigs, but it did not stimulate agglutinins in these animals and this was taken as evidence that the cattle had not been infected. The evidence is sufficient to show that the strain was of reduced virulence for cattle. Some of the cattle vaccinated with this strain together with controls were exposed to infection by feeding and by dropping *Br. abortus* on to the eye, and others were exposed to an eye infection only. The eye infection was heavy, consisting of three to four drops of a suspension equivalent to 20 times McFarland

tube 1. The results were as follows : of seven vaccinated animals infected by feeding and by dropping culture on to the eye three calved normally, two gave birth to weak calves and two aborted ; four of the animals had a uterine infection and the same four animals were infected in the udder. Of seven controls one calved normally and six aborted ; six showed a uterine infection, the same animals also being infected in the udder. Of three vaccinated animals infected by dropping culture on to the eye, one calved normally, one gave birth to a weak calf and one aborted ; the uterus and udder were infected in the two latter animals. Of the two controls in this series one gave birth to a weak calf and one aborted ; both animals were infected in the uterus and in the udder.

These results give the suggestion, although in a manner that is far from convincing, that the attenuated vaccine had given some immunity which might have been more clearly shown had smaller infective doses been used.

Cotton, Buck and Smith (1933) vaccinated three groups of cattle with three different living vaccines two months before the animals were served, and later exposed the vaccinated animals and controls to infection by placing on the eye three to four drops of a suspension of a culture of *Br. abortus* equivalent in opacity to 20 times tube 1 of the McFarland scale. One group was vaccinated with a culture (strain 11) that had been cultivated in the laboratory for twelve years and this strain was said not to cause macroscopic lesions in inoculated guinea-pigs. The second group was vaccinated with a culture (strain 19) that had been cultivated for six years and produced lesions in some but not all of the guinea-pigs that were inoculated with it. The third group received a virulent culture (strain 484). From the authors' discussion on the experiment it is clear that both strains 11 and 19 were only relatively non-virulent as it is admitted that even strain 11 caused moderately marked lesions demonstrable in guinea-pigs killed two months after infection. Furthermore, pregnant susceptible cows that received subcutaneous inoculations of vaccine prepared from this strain in some cases " maintained serological reactions of such intensity as to indicate localisation of infection in their udders." The authors state : " As the experiment proceeded and further guinea-pig inoculation tests were made of strains 11 and 19, some doubt arose as to whether there was a considerable degree of difference between the virulence of these two strains." The fact that neither of these strains could be regarded as non-virulent for either guinea-pigs or cattle is stressed, particularly as further reference to work with strain 19 will be quoted. The results of the vaccination experiment were as follows :—

Six of seven animals vaccinated with strain 11 calved normally and one died at parturition. *Br. abortus* was recovered from the uterus of the latter animal and from the uterus of one that calved normally. Eight animals were vaccinated with strain 19 and all calved normally, but *Br. abortus* was isolated from the uterus of three of these. Eight animals were vaccinated with the virulent strain 484 and seven of them calved normally ; one had a weak calf and *Br. abortus* was isolated from the uterus of this cow but not from the others. Eight controls were used and seven of them aborted ; *Br. abortus* was isolated from the uterus of all eight. Very considerable resistance to infection was shown by all the vaccinated groups although the infective dose was heavy.

The last reference that need be quoted in any detail refers to further work on the immunisation of cattle with strain 19 carried out by Haring and Traum (1937). Their report shows that the strain is by no means non-virulent for cattle on subcutaneous inoculation. Thus the strain was recovered

from the uterus and udder of two out of six cows inoculated when pregnant. Cattle were vaccinated at different ages and exposed to infection during pregnancy at different periods after vaccination. Some vaccinated and control cattle were exposed to infection by applying 0·4 c.c. of a suspension of *Br. abortus* to the eye and then massaging the closed eyelids. It was estimated that the quantity of suspension administered to each animal contained 1,500 million living bacteria. Other animals received an infective dose in the same manner but estimated to contain 10,000 million bacteria. It is shown that of eleven vaccinated cattle given the smaller infective dose, eight produced normal calves and three aborted. The former showed no *Brucella* infection, but *Br. abortus* was recovered from the uterus of the three latter animals. Of 13 controls only one gave birth to a normal calf and was not infected, the other twelve aborted and were infected. The larger infective dose caused a higher rate of infection among the vaccinated cattle. Four of nine pregnant animals aborted and one gave birth to a weak calf, all five being infected; the four remaining animals were not infected. Of 13 controls only two calved normally and 12 were infected. The authors suggest that had the smaller infective dose been adhered to there would have been less infection among the vaccinated animals.

It will be seen from this brief review of the literature that immunity following vaccination with strains of very low virulence is not of a high order, and there is reason to think that strains of greater but still reduced virulence confer a higher resistance, but even such a resistance may not be so great as that given by vaccination with fully virulent cultures. It is, however, extremely difficult, if not impossible, to make definite comparisons of any two experiments for two reasons. One is that cattle show a great variation in their response to infection and often the numbers of animals used have been so small that a natural variation in susceptibility may have influenced the final results very materially. The other important reason is the impossibility of forming any comparison of the exposures to infection used in different experiments. These must have varied greatly, although it is probable that they were always on the severe side. The possibility of a severe infective dose overwhelming the immunity conferred by the vaccines of the lower degrees of virulence is indicated in the experiments referred to, but the probability that vaccines of this type may confer a serviceable immunity on non-infected animals in the field is evident from the favourable results of field experiments reported by Meyer and Huddleson (1936) and McEwen (1937). The desirability of an infective dose that would not overwhelm an immunity but would demonstrate it is obvious.

If, as we believe, it is possible to maintain strains of *Br. abortus* over indefinite periods of time without the occurrence of changes of virulence or of antigenic structure, and if the same infective dose or doses were used at different times, it would be possible in carrying out any one vaccination experiment to spread the work and expense over a period of time and so to use a sufficient number of animals to eliminate incorrect interpretations owing to chance error.

Experimental Procedure.

The strain that was used was isolated from a bovine fœtus on March 23rd, 1936. The second generation of this strain from a liver agar slant was preserved by drying *in vacuo* over P_2O_5 and the second subcultures from the preserved strain were used for infection experiments on cattle, and for infection experiments on guinea-pigs that have been repeatedly made over a period of two years. In all the infection experiments 48 hour cultures on liver agar were used. The surface growth was washed off with saline and for the infection experiments on guinea-pigs standardised to No. 3 tube Brown's scale. Dilutions in multiples of ten were made from this suspension, a separate pipette being used for each dilution. Groups of guinea-pigs, containing not less than twelve animals per group, were inoculated intramuscularly and other groups were infected by the application of a small quantity of the different suspensions to the eye. The animals were killed six weeks after infection; their sera were tested for agglutinins and cultures were made from their spleens. The results of some of these examinations are summarised in Table I. It will be seen that over the period tested the strain underwent no detectable deterioration in virulence, the guinea-pig infection experiments being satisfactorily constant in their results. These results we regard as typical for field strains of *Br. abortus* isolated in this area of the country from cattle. We have not found any evidence that freshly isolated CO_2 sensitive field strains vary materially in virulence when the tests include a sufficient number of guinea-pigs.

Table I.

Showing the Percentage of Guinea-pigs in the different Groups found with Spleens Infected with *Br. abortus* Six Weeks after Exposure to Infection with Strain 544.

Methods of Exposure.	*Dilutions of Suspensions of Organisms Equivalent to Brown's No. 3 Tube.*	*Dates of Inoculation.*						
		10.9.36	12.1.37	12.3.37	12.7.37	29.11.37	7.3.38	23.9.38
Intramuscular inoculation of 1 c.c.	1 : 10^5	100	100	.	100	.	100	.
	1 : 10^6	100	90	.	100	100	100	100
	1 : 10^7	100	80	.	83	100	100	100
	1 : 10^8	0	78	.	58	83·3	40	100
	1 : 10^9	.	.	.	.	.	0	.
Drop on eye	Undiluted	.	.	100	100	.	100	.
	1 : 10	.	.	100	100	90	90	.
	1 : 10^2	.	.	60	70	90·9	75	.
	1 : 10^3	.	.	50	27	12·5	8·3	.
	1 : 10^4	.	0	0	1	.	0	.

The cattle that were used were purchased in the open market as non-pregnant heifers in May, 1937, and throughout the experiment

were kept on sheep pastures isolated from other cattle on the Romney Marsh, Kent. Agglutination tests were made on the heifers within a few days of purchase and at intervals until the animals were exposed to infection. None of the animals showed any agglutinins at titres of 1 : 10 and upwards until after infection. On September 20th, 1937, a bull was run with the heifers and a few weeks later this animal was replaced by a fresh bull. The opportunities for service were so arranged that the animals should calve during the summer when attendance on calving or aborting animals kept out of doors would be much easier than at other times of the year. The correct service dates for all but a few of the animals were obtained.

In March, 1938, the animals were divided into five groups, ten animals in each. In allocating the animals to the different groups care was taken to ensure that each group contained an equal number of animals at approximately the same stage of pregnancy. The decision which infective dose was to be given to each group was decided by random selection.

The groups having been made, each was allotted to a fresh isolated field, adequately fenced and in each field a railed-in pound for collecting the animals was built. The animals were exposed to infection on March 7th, 1938. Suspensions of *Br. abortus* strain 544 were prepared as described and standardised to tube 10 on the Brown's scale. Each animal was given an infective dose by dropping 0·1 c.c. of suspension on to the eye. The eye was not manipulated in any other way.

Viable counts were made in the usual way on the suspension used and according to the counts the number of bacteria given to each animal in the respective groups were as follows:—Group 1 received 1,460,000,000; group 2 received 14,600,000; group 3 received 1,460,000; group 4 received 146,000 and group 5 received 1,460.

The multiple of ten intervenes between the numbers of organisms given to each of groups 2, 3 and 4. Group No. 1, however, received a dose 100 times larger than that given to group No. 2, as we wished to give what was thought would be a certain infective dose to this group. On the other hand, group 5 received a dose 100 times less than that given to group 4 in the anticipation that by so doing something less than the infective dose would be given.

The animals were bled and their sera tested for agglutinins at intervals. The fœtus and membranes were obtained whenever possible, but in the event of the membranes being eaten uterine swabs were collected. These, together with colostrum, were examined bacteriologically for *Br. abortus*. The colostrum was also tested for agglutinins.

In carrying out the bacteriological examinations cultures were made from the stomach contents, spleen, kidneys, and liver of fœtuses, and stomach contents were inoculated into each of at least four guinea-pigs. In cases of abortion, emulsions prepared from the membranes were also inoculated into guinea-pigs if smears made from

TABLE II.

SUMMARISING THE EFFECTS OF INOCULATING HEIFERS WITH VARIOUS NUMBERS OF VIABLE *Br. abortus* ORGANISMS.

Heifer Number.	*Number of Viable Organisms. Inoculated.*	*Days from*		*How Pregnancy Ended.*	*Result of Attempt to Isolate Br. abortus.*
		Service to Infection.	*Infection to End of Pregnancy.*		
Group 1					
1	1460×10^6	163	32	A	+
2	„	143	32	A	+
3	„	151	Not in calf		
4	„	136	51	A	+
5	„	156	54	A	+
6	„	161	59	A	+
7	„	163	59	A	+
8	„	Not known	59	A	+
9	„	153	129	C	+
10	„	60	226	C	+
Group 2					
11	1460×10^4	148	37	A	+
12	„	149	40	A	+
13	„	Not known	43	A	+
14	„	119	45	A	+
15	„	131	50	A	+
16	„	164	70	A	+
17	„	151	70	A	+
18	„	158	86	A	+
19	„	Not known	98	P	+
20	„	„	193	C	—
Group 3					
21	1460×10^3	150	79	A	+
22	„	156	88	A	+
23	„	162	108	P	+
24	„	Not known	115	C	—
25	„	166	119	C	—
26	„	133	119	A	+
27	„	153	122	C slightly P	—
28	„	106	125	P	+
29	„	159	130	C	+
30	„	144	141	C	+
Group 4					
31	1460×10^2	157	83	A	+
32	„	163	122	C	—
33	„	147	88	A	+
34	„	Not known	125	C	—
35	„	163	125	C	+
36	„	152	Approx. 122	Probably P	+
37	„	148	131	C	—
38	„	108	151	P	+
39	„	Not known	195	C	—
40	„	97	Not in calf		
Group 5					
41	1460	162	118	C	—
42	„	161	118	C	—
43	„	165	121	C	—
44	„	158	124	C	—
45	„	154	128	C	—
46	„	150	130	C	+
47	„	136	146	C	—
48	„	105	179	C	—
49	„	108	184	C	+
50	„	59	Not in calf		

A = aborted. P = premature. C = calved. + = *Br. abortus* isolated.
— = *Br. abortus* not isolated.

TABLE III.

SHOWING THE APPEARANCE OF THE PLACENTAE TOGETHER WITH ATTEMPTS TO ISOLATE *Br. abortus* FROM THE PLACENTAE, FOETUSES AND COLOSTRUM.

Heifer Number.	*Placenta.* Macroscopic Appearance.	*Placenta.* Smears.	*Placenta.* Guinea-pigs.	*Fœtus.* Cultures. Stomach.	*Fœtus.* Cultures. Liver.	*Fœtus.* Cultures. Kidney.	*Fœtus.* Cultures. Spleen.	*Fœtus.* Guinea-pigs.	*Colostrum.* Culture.	*Colostrum.* Guinea-pigs.
Group 1										
1	Typical necrosis	+	.	+	+	+	+	+	+	+
2	Placenta lost	.	+*	Fœtus found putrid					+	+
3		Not in calf								
4	Typical necrosis	+	.	+	+	+	+	+	+	+
5	„ „	+	.	+	+	+	+	+	+	+
6	„ „	+	.	+	+	+	+	+	+	+
7	„ „	+	.	+	+	+	+	+	+	+
8	„ „	+	.	+	+	+	+	+	+	+
9	Normal	—	—	.	.	.	.	.	+	+
10	„	—	+	.	.	.	.	.	—	+
Group 2										
11	Typical necrosis	+	.	+	+	+	+	+	+	+
12	„ „	+	.	+	+	+	+	+	+	+
13	„ „	+	.	+	+	+	+	+	+	+
14	„ „	+	.	+	+	+	+	+	+	+
15	„ „	+	.	+	+	+	+	+	+	+
16	„ „	+	.	+	+	+	+	+	+	+
17	„ „	+	.	+	—	—	+	+	—	—
18	Slight „	?	.	+	+	—	+	+	+	+
19	Typical „	+	+	.	.	.	.	.	+	+
20	Placenta lost	—	—*	.	.	.	.	.	.	—
Group 3										
21	Typical necrosis	+	+	+	+	+	+	+	+	+
22	„ „	+	+	+	+	+	+	+	—	+
23	Slight „	+	+	.	.	.	.	.	—	+
24	Normal	—	—	.	.	.	.	.	—	—
25	„	—	—	.	.	.	.	.	—	—
26	Slight necrosis	+	.	+	—	c	+	+	—	+
27	Necrosis of cotyledons	.	—	.	.	.	.	.	—	—
28	Typical necrosis	+	+	.	.	.	.	.	—	+
29	„ „	+	+	.	.	.	.	.	+	+
30	Placenta lost	—*	+*	.	.	.	.	.	+	+
Group 4										
31	Typical necrosis	+	.	+	—	—	+	+	+	+
32	Normal	—	—	.	.	.	.	.	.	—
33	Typical necrosis	+	.	c	+	+	+	+	—	—
34	Placenta lost	.	—*	—	c	—	—	—	—	—
35	Normal	—	+	.	.	.	.	.	—	—
36	Placenta lost	—*	—*	Calf found drowned & putrid					+	+
37	Normal	—	—	.	.	.	.	.	—	—
38	Typical necrosis	+	+	.	.	.	.	.	—	+
39	Normal	—	—	.	.	.	.	.	—	—
40		Not in calf								
Group 5										
41	Normal	—	—	.	.	.	.	.	—	—
42	„	—	—	.	.	.	.	.	—	—
43	„	—	—	.	.	.	.	.	—	—
44	Placenta lost	—*	—*	.	.	.	.	.	.	—
45	Normal	—	—	.	.	.	.	.	—	—
46	„	—	+	.	.	.	.	.	—	—
47	Placenta lost	.	—*	.	.	.	.	.	—	—
48	„ „	—*	—*	.	.	.	.	.	—	—
49	Normal	—	+	—	—	—	—	—	—	—
50		Not in calf								

* = Vaginal or uterine material. + = *Br. abortus* present. ? = Doubtful
— = *Br. abortus* absent. . = Not done. c = ? ? ?

the fœtal stomach contents failed to show *Br. abortus* at microscopical examination. A note was made of the extent of the macroscopic lesions shown by the membranes and smears were prepared from the cotyledons. When living calves were born the membranes were examined in a similar manner but material from each set of membranes was inoculated into eight guinea-pigs. The colostrum was allowed to gravitate out for a few hours or overnight and material from the upper layers plated on fuchsin agar and on malachite green thionin blue agar (Stockmeyer, 1933); 3 c.c. of this material was also inoculated intramuscularly into each of four guinea-pigs.

The results of these examinations are summarised in Tables II, III and IV.

From the results shown in Table II it will be seen that approximately $1{,}460 \times 10^6$ viable *Br. abortus* organisms infected all the nine pregnant animals exposed, causing seven of these to abort. In the second group, $1{,}460 \times 10^4$ organisms infected nine of ten animals, causing eight of these to abort and one to calve prematurely. In the third group, receiving $1{,}460 \times 10^3$ organisms, seven of the ten animals were infected and five of them aborted or calved prematurely. In the fourth group, receiving $1{,}460 \times 10^2$ organisms, five of ten animals were infected and three of these aborted or calved prematurely. In the final group of ten animals receiving only 1,460 organisms, two showed a very slight infection but all of them calved normally.

It may be noted that the percentages of infection, abortion and premature calving among the animals receiving $1{,}460 \times 10^4$ organisms were as high as those which have generally been obtained by other workers when much larger doses have been given by natural routes.

In selecting an appropriate infective dose to test the immunity of vaccinated cattle the two factors which should receive chief consideration are the percentage of abortions or premature calvings and the percentage of infections that are likely to be caused. The information given by this experiment with limited numbers of animals when considered along with the results obtained by others in immunisation tests gives some guidance which was formerly lacking regarding an appropriate dose for testing the immunity of cattle.

It is probable that animals vaccinated with living virulent strains of *Br. abortus* would show a high degree of resistance towards the largest dose tested, namely $1{,}460 \times 10^6$ organisms. It would appear, however, that the second largest dose of $1{,}460 \times 10^4$ organisms constitutes a severe exposure to infection and one that may be expected to infect a high percentage of animals. Although a dose of this size is probably much smaller than any that has so far been used to test the immunity of cattle it is sufficiently severe for this purpose; even if small numbers of vaccinated cattle were used and they withstood infection or showed a definitely greater resistance to this dose than did controls, it would afford evidence of immunity. If a less drastic test was required to assess the resistance of animals possessing a

TABLE IV.

SHOWING THE AGGLUTININ TITRES OF HEIFERS AT VARIOUS TIMES AFTER EXPOSURE TO *Br. abortus* INFECTION.

Heifer Number.	Days after Exposure to Infection.									
	14	28	46	65	80	106	123	156	205	227
Group 1										
1	160	80	640	1,280						
2	160	160	2,560	640						
3	40	80	180	160						
4	0	40	160	5,120						
5	160	40	80	2,560						
6	40	160	320	2,560						
7	160	80	1,280	1,280						
8	20	80	40	160						
9	40	40	40	80		80	40			
10	0	40	20	0	0	0	10	0	40	20
Group 2										
11	0	40	640	1,280						
12	0	80	320	320						
13	0	10	40	2,560						
14	0	10	80	160						
15	0	20	20	640						
16	0	10	20	20						
17	0	20	40	5,120						
18	0	0	20	40	1,280					
19	0	40	20	10	320					
20	20	0	0	0		0	0	0	0	
Group 3										
21	0	0	0	0	80					
22	0	10	20	20						
23	0	0	0	0	10	320				
24	0	0	0	0	0	0	0			
25	0	0	0	0	0	0	0			
26	0	0	0	10	20	80				
27	0	0	0	0	0	0	0			
28	0	0	10	0	10	10	640			
29	0	0	0	0	10	320				
30	0	0	0	0	0	0	0			320
Group 4										
31	0	0	10	160			640			
32	0	0	0	0	0	0	0			
33	0	0	0	0	320					
34	0	0	0	0	0	0	0		0	
35	0	0	0	0	0	0	0			
36	0	0	0	0	0	40	80			
37	0	0	0	0	0	0	0		0	
38	0	0	0	0	0	0	0	640		
39	0	0	0	0	0	0	0	0	0	
40	0	0	0	0						
Group 5										
41	0	0	0	0	0	0	0			
42	0	0	0	0	0	0	0		0	0
43	0	0	0	0	0	0	0		0	0
44	0	0	0	0	0	0	0		0	
45	0	0	0	0	0	0	0		0	
46	0	0	0	0	0	0	10 (10 at 143 days)			
47	0	0	0	0	0	0	0		0	
48	0	0	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0	40	160
50	0	0	0	0	0	0	0	0		

relatively low grade immunity obviously a smaller dose could be used, provided sufficient animals were employed to meet the requirements of statistical accuracy. In immunisation tests it would probably always be an advantage to test the resistance of groups of immunised animals with two or more selected doses.

Attention may be directed to other points of interest shown by the experiment. One of these is the interval elapsing between infection and the demonstration of agglutinins in the sera of the animals in the different groups. This interval increases inversely as the number of infecting organisms. Thus eight of ten animals in group 1 showed agglutinins 14 days after infection, whereas at that time only one animal in group 2 and none in any of the other groups showed agglutinins ; the last two groups still remained negative. Forty-six days after infection all the nine animals in group 2 that finally became infected were showing agglutinins, whereas only two animals in group 3, one in group 4 and none in group 5 showed agglutinins. Agglutinins were not demonstrated in the two animals which became infected in group 5 until 100 to 200 days after exposure ; it is to be noted that we regard it as unlikely that these two animals became infected from any other source than the inoculum, since care was taken to avoid any chance of spread from the other animals in the experiment and no other cattle were kept in the vicinity.

Another matter of interest is the relation between the period that intervened between infection and abortion and the extent of the lesions shown by the fœtal membranes. In the cases of abortion that occurred less than 60 days after infection, confined in groups 1 and 2, the fœtal membranes were most extensively diseased ; they showed a more advanced and extensive necrosis than that which is found, except very rarely, in the fœtal membranes from animals that have aborted in the field. This observation indicates that the infective doses given to the animals in groups 1 and 2 were more severe than those to which animals are generally exposed in the field and that animals resistant to such doses might be immune to natural infection.

Summary.

The experiment outlined in this paper was designed to estimate a suitable number of viable *Br. abortus* organisms which might be used to test the immunity of vaccinated cattle. The necessity for estimating this dose is discussed.

References.

Birch, R. R., and Gilman, H. L. (1922). Rept. New York State Vety. Coll., 1922-23 ; (1925). *Cornell Vet.*, **15,** 90 ; (1926). Rept. New York State Vety. Coll., 1926-27.

Buck, T. M., and Creech, G. T. (1924). *J. Agric. Res.*, **26,** 607.

Cotton, W. E. (1932). *Ibid.*, **45,** 705.
——— Buck, J. M., and Smith, H. E. (1933). *Ibid.*, **46,** 291.
——— and ——— (1933). *Ibid.*, **46,** 315.
Haring, C. H., and Traum, I. (1937). *Ibid.*, **55,** 117.
Hart, G. H., and Traum, I. (1925). Univ. Calif. Coll. Agric. Expt. Stn. Tech. Paper No. 19.
McEwen, A. D. (1937). *Vet. Rec.*, **49,** 1585.
——— and Roberts, R. S. (1936). *J. Comp. Path. & Ther.*, **49,** 97.
——— and Priestley, F. W. (1938). *Vet. Rec.*, **50,** 1097.
M'Fadyean, J., Sheather, A. L., and Minett, F. C. (1913). *J. Comp. Path. & Ther.*, **26,** 142.
——— and Stockman, S. (1909). Report of Departmental Committee on Epizootic Abortion, London. Appendix to Pt. 1.
Meyer, D. B., and Huddleson, I. F. (1936). Bull. No. 153, Mich. Agric. Expt. Stn.
Rettger, L. F., McAlpine, J. G., White, G. C., and Johnston, R. E. (1926). Bull. No. 137, Storrs Agric. Expt. Stn.
Stockmayer, W. (1933). *Zbl. Bakt. Orig.*, **128,** 205.

THE DEMONSTRATION OF IMMUNITY IN CATTLE VACCINATED WITH A NON-VIRULENT STRAIN OF *BR. ABORTUS**

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In the preceding paper by McEwen, Priestley and Paterson (1939) experiments are reported on the determination of a suitable infective dose of *Br. abortus* that could be used to test the immunity of vaccinated cattle, and it was suggested that $1{,}460 \times 10^4$ organisms of the strain tested constituted a sufficiently severe dose for this purpose. The present paper records an experiment in which the immunity of two small groups of vaccinated cattle was tested and provides confirmatory evidence regarding the suitability of an infective dose of $1{,}460 \times 10^4$ organisms applied to the surface of the eye.

Methods.

The ten cattle used in an earlier experiment to test the innocuousness of *Br. abortus* strain 45 (McEwen, 1937) were each inoculated subcutaneously on September 13th, 1937, with 8 c.c. of a suspension of strain 45 prepared by washing off the surface growth from 48-hour liver agar slant cultures and standardising to an opacity equivalent to No. 6 tube Brown's scale. The inoculation was repeated on November 4th, 1937, and on January 14th, 1938, fresh suspensions being made on each occasion. All except one of the animals had been served by the time the second inoculation was given and this latter animal was served before the third inoculation was made.

On March 7th, 1937, the cattle were given an infective dose of *Br. abortus* strain 544 ; five animals each received a dose of $1{,}460 \times 10^6$ organisms and the remainder each received a dose one hundred times smaller, namely $1{,}460 \times 10^4$ organisms. The actual suspensions of culture of strain 544 used were those employed for infecting the animals in groups 1 and 2 in the previous experiment and the exposure to infection was the same, namely the application of 0·1 c.c. of the suspension used to the surface of the eye. The animals in groups 1 and 2 of the previous experiment therefore acted as controls to the vaccinated cattle. The bacteriological and other examinations on material from the vaccinated cattle were made in the same way as those recorded in the preceding experiment. In all cases when

* This work was carried out under the general co-ordination of the Committee of the Agricultural Research Council on *Br. arbortus* Infection in Animals and Man, with the aid of funds provided on the recommendation of the Council, including funds supplied by the Milk Marketing Board.

Br. abortus was isolated the organisms recovered were found to be CO_2-sensitive showing, that the CO_2-sensitive strain used for the infective dose and not the aerobic vaccine strain had been isolated.

The results of the examinations on the vaccinated animals are recorded in Tables I and II and in Table III comparable data for the vaccinated and control animals are summarised.

TABLE I.

SUMMARISING THE EFFECTS OF INOCULATING VACCINATED CATTLE WITH TWO DIFFERENT SIZED DOSES OF *Br. abortus* ORGANISMS.

Animal Number.	*Number of Viable Organisms Inoculated.*	*Days from*		*How Pregnancy Ended.*	*Results of Attempts to Isolate Br. abortus.*
		Service to Infection.	*Infection to End of Pregnancy.*		
1	1460×10^6	166	63	A	+
2	„	142	97	P	+
3	„	148	117	P	+
4	„	59	232	C	—
5	„	217	51	P	+
6	1460×10^4	151	99	P	+
7	„	156	105	P	+
8	„	153	125	C (twins)	—
9	„	150	132	C	—
10	„	154	126	C (twins)	—

A = Aborted. P = Premature. C = Calved. + = *Br. abortus* isolated.
— = *Br. abortus* not isolated.

TABLE II.

SHOWING THE APPEARANCE OF THE PLACENTA TOGETHER WITH ATTEMPTS TO ISOLATE *Br. abortus* FROM THE PLACENTAE, FOETUSES AND COLOSTRUM.

Animal Number.	*Placenta.*			*Fœtus.*					*Colostrum.*	
				Cultures.						
	Macroscopic Appearance.	*Smears.*	*Guinea-pigs.*	*Stomach.*	*Liver.*	*Kidney.*	*Spleen.*	*Guinea-pigs.*	*Culture.*	*Guinea-pigs.*
1	Lost	+*	+	+	—	—	+	+	+	+
2	Slight necrosis	+	+	C	—	—	—	+	+	+
3	Typical „	+	+	.	.	.	.	.	+	+
4	Normal	—	—	.	.	.	.	.	—	—
5	Slight necrosis	+	.	+	—	+	+	+	—	+
6	Very slight necrosis	+	.	—	—	+	—	+	—	—
7	„ „	+	+	.	.	.	.	.	+	+
8	Normal	—	—	.	.	.	.	.	—	—
9	„	—	—	.	.	.	.	.	—	—
10	„	—	—	.	.	.	.	.	—	—

* = Vaginal or uterine material. + = *Br. abortus* present. — = *Br. abortus* absent.
. = Not done. C = Contaminated.

Table III.

Summarising the Results Shown by the Vaccinated and Control Animals after Exposure to Infection.

Dose. Number of Organisms.	*Number of Animals.*	*Abortions or Premature Calvings.*	*Normal Calvings.*	*Number Infected.*	*Number not Infected.*
1460×10^6	Vaccinated 5	4	1	4	1
„	Controls 9	7	2	9	0
1460×10^4	Vaccinated 5	2	3	2	3
„	Controls 10	9	1	9	1

Tables I and II show that the larger infective dose caused the more severe infection of the vaccinated animals. In the experiment where normal cattle were used (see McEwen, Priestley & Paterson, 1939) the increased severity of the larger of two infective doses was not manifest. It is apparent that vaccination had altered the reactivity of the animals, the vaccinated animals showing an increased resistance to the smaller infective dose. The resistance of the vaccinated cattle was, however, insufficient to differentiate them from control animals when the size of the infective dose was increased one hundred fold. The experiment provides evidence showing that cattle inoculated with living cultures of a non-virulent strain of *Br. abortus* that causes no infection, possess an increased resistance to infection that can be demonstrated by testing with an infective dose that is not too severe.

References.

McEwen, A. D. (1937). *Vet. Rec.*, **49,** 1585.
———, Priestley, F. W., and Paterson, J. D. (1939). *J. Comp. Path. and Ther.*, **52,** 116.

A SIMPLE CONFIRMATORY MEDIUM FOR *STR. AGALACTIAE*

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The first essential in any scheme for the eradication of contagious bovine mastitis from a herd is the bacteriological examination of the milk from each quarter of every cow in the herd. Davis and McClemont[1] have shown that the indirect tests are quite unreliable and have emphasised the importance of simple methods in the plating of milk samples. When non-blood media are used it is essential to pick off suspected colonies for confirmation by biochemical or serological tests. Even when blood media are used, it is advisable to carry out this procedure in all but the very obvious cases, *i.e.*, with plates containing numbers of small light-coloured colonies surrounded by zones of " weak β " hæmolysis.[2]

The most characteristic properties of *Str. agalactiæ* are (i) hydrolysis of hippurate[3], (ii) acid and clot without reduction in yeast litmus milk[4], (iii) non-fermentation of æsculin[3, 5] and (iv) orange deposit in starch broth cultures[6].

Of these, the first and fourth are probably the most peculiar to *Str. agalactiæ*. While it would be possible to combine these tests in one medium, it may be observed that testing for hippurate hydrolysis is a matter requiring some skill and also that the formation of the orange pigment with starch is only evident in about 80 per cent. of strains examined ([2]). The remaining two properties are, as far as we have observed, constant. A medium has been devised to permit the determination of these reactions in a simple and convenient manner.

Composition of medium.

Separated milk	1,000 ml.
1 per cent. litmus solution[7]	10 ml.
Autolysed yeast[7]	10 ml.
Æsculin	5 g.
5·8 per cent. ferric chloride solution (commercial 58 per cent. diluted 10 times).	1 ml.

Adjust to *p*H 7·0, fill out into tubes and sterilise by intermittent steaming or momentary autoclaving.

Procedure.

Any medium permitting the growth of *Str. agalactiæ* may be used for the plating provided that this is carried out within three hours of milking. (cf. [2, 8, 9].) Well-isolated colonies presumed

to be *Str. agalactiæ* are picked off into tubes of the medium, incubated at 37° C. for 48 hours and then examined. At least three whole colonies should be picked off.

Diagnosis.

Acidity and clotting without reduction of the litmus and without darkening of the medium may be taken as presumptive evidence of *Str. agalactiæ* (Table 1).

This characteristic reaction is produced by *Str. agalactiæ* and also by some non-æsculin fermenting strains of *Str. pyogenes*. The latter are rare in properly taken udder samples and even if diagnosed as *Str. agalactiæ* in eradication schemes no harm is done, as cows harbouring such streptococci should rightly be regarded as undesirable. The use of blood media for the plating usually permits the differentiation of *Str. pyogenes* from *Str. agalactiæ*. *Str. thermophilus* produces a similar reaction in this medium but is not known to occur in udder samples.

Remarks.

Ferric chloride has two functions in the medium. (1) To give a black colour with the decomposition products of æsculin (æsculetin) and (2) to inhibit the slight tendency of some strains of *Str. agalactiæ* to reduce litmus in yeast litmus milk. It has been found, in fact, that all organisms which normally bring about a slight reduction of litmus in milk no longer do so when ferric chloride is incorporated in the medium. It is possible to control the extent of reduction by varying the concentration of ferric chloride. The addition of the yeast autolysate ensures rapid and certain growth of all colonies of *Str. agalactiæ*.

TABLE 1.

Organism	*Acidity*	*Clot*	*Reduction*	*Darkening*	*Catalase*
Str. agalactiæ ...	+	+	0	0	0
Str. bovis	+	±	0	+	0
Str. lactis	+	+	++	+	0
Str. fæcalis	+	±	++	+	0
Str. pyogenes ...	+	±	0	±	0
Str. salivarius ... (" viridans ")	+	+	++	+	0
Minett Group II[3]	+	+	++	+	0
„ „ III[3]	+	+	+	0	0
Udder cocci ... (lactose fermenting)	+	+	+	±	+
Udder cocci ... (non-lactose fermenting)	0	0	0	0	+

Reduction: + = partial reduction of litmus; ++ = complete reduction of litmus.

In Table 1 are given the appearances of the medium after growth of organisms likely to occur in udder samples.

Udder cocci are readily differentiated by the catalase test. This may be carried out by adding 1 ml. of 10 vol. hydrogen peroxide to the culture or by adding 1 ml. of the culture to c. 3 vol. hydrogen peroxide preferably in phosphate buffer at *p*H 6·8. A steady evolution of small bubbles indicates a catalase-positive organism, probably a " micrococcus." All streptococci are catalase negative.

References.

[1]Davis and McClemont. (1939). J. Dairy Res., **10,** 74, 81, 88 and 94.
[2]Davis, McClemont and Rogers. (1939). *Ibid.*, **10,** 59.
[3]Minett. (1936). *J. Hyg.*, **35,** 504.
[4]Davis. (1938). *J. Dairy Res.*, **9,** 85.
[5]Orla Jensen, A. D. (1934). *Acta. Path. Microbiol. Scand.*, **11,** 312.
[6]Orla Jensen, S. (1919). " The lactic acid bacteria," Copenhagen.
[7]Davis. (1935). *J. Dairy Res.*, **6,** 121.
[8]Edwards. (1933). *J. Comp. Path.*, **46,** 211.
[9]Klimmer and Weiske. (1937). *Milch. Forsch.*, **19,** 15.

CERVIX BIFIDA IN THE DOMESTIC COW (*BOS TAURUS*).

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Introduction.

During the course of research work at the Institute of Animal Pathology, Cambridge, which necessitated the use of a number of uteri obtained from cattle killed in the local slaughterhouses, an interesting abnormality of the genital tract was encountered. The cervical canal was double in its caudal part, the vagina and corpus uteri being single. Within a short period a comparable specimen was found and it was then decided to enquire into the relative frequency of the condition. Accordingly, the cervices of 72 cattle slaughtered in the Glasgow City Abattoir were examined. Of these one was certainly from a freemartin, and another probably so; these were discarded as unsuitable material. Of the remaining 70 all had a single cervical canal except one, which was similar to the second specimen obtained in Cambridge.

Description of Specimens.

None of the animals furnishing these specimens was seen alive.

Case 1. The complete genitalia of this heifer were obtained together with the history that she had been mated at least twice and on being found non-pregnant had been sold for slaughter.

External examination of the organs revealed no abnormality, but it was found on dissection that there were two ora uteri externa. Introduction of probes into the left and right openings showed that the canals leading from them communicated cranially with each other.

The specimen was dissected from the dorsal aspect and the appearance reproduced in Plate I was the result.

For the purpose of demonstrating the condition a coloured probe was laid along the course of each cervical canal. It will be seen that the left hand probe has been laid a little external to the actual canal; this was done with the intention of emphasising the contour of the passage. Plate II is included to show the normal single nature of the corpus uteri and the cephalic portion of the cervix uteri. Here the left hand probe has been moved medialwards and laid in the left cervical canal.

Case 2. No history of this animal was available.

The condition was comparable to that in Case 1, but here the duplicity was less extensive so that in the fresh state a probe could be introduced through one os uteri externum and passed back out through the other. The relative appearance of the cervices in Cases 1 and 2 are shown schematically (Fig. 1).

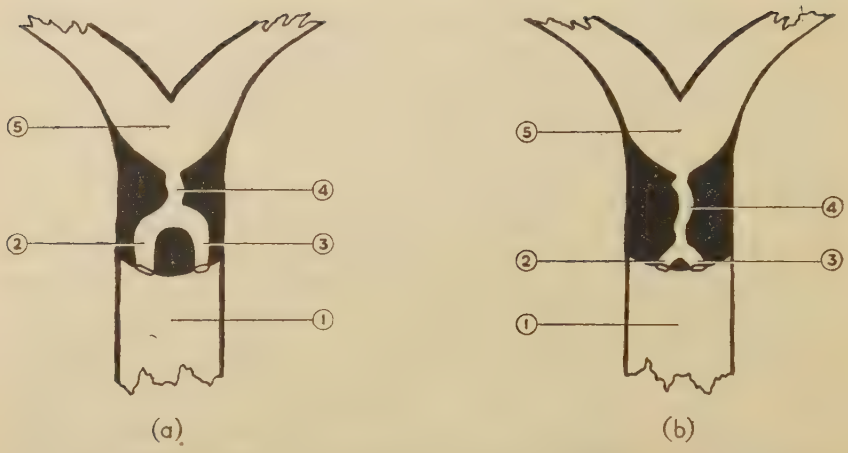

FIG. 1. Diagram showing the approximate degree of duplicity in Cases 1 and 2. (a) Cervical region in Case 1. (b) Cervical region in Case 2. 1. Vagina. 2. Left cervical canal. 3. Right cervical canal. 4. United cervical canal. 5. Corpus uteri.

Case 3. This case was a two-year-old heifer which had evidently never been pregnant and the organs were similar to those in Case 2.

We have, therefore, three comparable cases with normal single corpus uteri, cervix uteri bifid in its caudal part, and normal single vagina. Any apparent differences in the degree of duplicity in these specimens appear to have been caused by a failure of the Müllerian ducts to fuse over a varying extent of one particular part of the genital tract, *viz.*, the cervical region. The occurrence of these abnormalities is explained on embryological grounds in a later discussion on the development of the female genital tract in Mammalia in general and in *Bos taurus* in particular.

SHORT SUMMARY OF THE DEVELOPMENT OF THE VAGINA AND UTERUS.

Among the lower vertebrates the left and right Müllerian ducts in the female do not fuse, so that the female genital apparatus consists of a bilaterally symmetrical set of tubes leading from the left and right ovary respectively to the urogenital sinus.

In the lowest order of Mammalia, the oviparous Monotremata, this condition persists (Fig. 2 (a)), the Müllerian ducts not being divided into uterine and vaginal segments.

The Marsupialia show an advance on this condition. They are viviparous and the Müllerian ducts are divided into uterine and vaginal portions. In most marsupials the right and left ducts do not fuse, but in some, *e.g.*, Macropodiæ, there is fusion in the region of the vaginal culs-de-sac resulting in the formation of a third, or median, vaginal canal.

PLATE I.

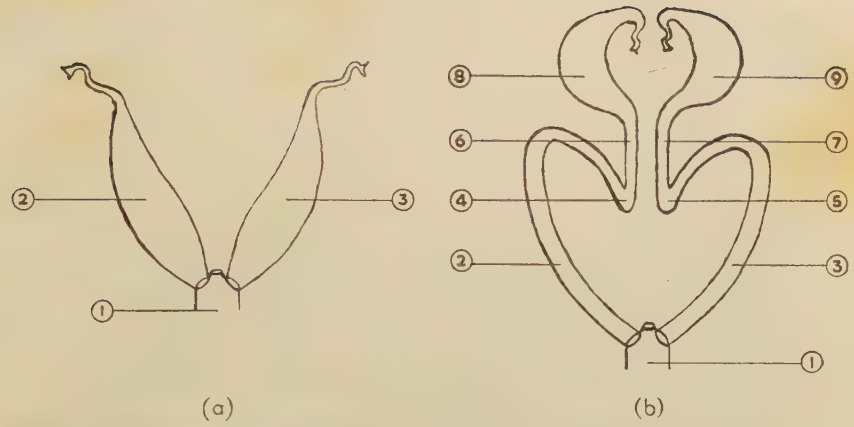

FIG. 2. Diagram showing primitive female genital apparatus in Monotremes and Marsupials.

(a) Monotremata, *e.g.*, *Ornithorhynchus anatinus*. 1. Urogenital sinus. 2. Left oviduct. 3. Right oviduct. (b) Marsupialia, *e.g.*,*Didelphys virginiana*. 1. Urogenital sinus. 2. Left vaginal canal. 3. Right vaginal canal. 4. Left vaginal cul-de-sac. 5. Right vaginal cul-de-sac. 6. Left cervix uteri. 7. Right cervix uteri. 8. Left uterus. 9. Right uterus.

The Müllerian ducts of the Eutheria fuse in the midline to a greater or less degree. This fusion commences at the caudal ends of the ducts and progresses cephalically. It is less marked in the lower forms and every gradation exists from the single vagina and Uterus bicornis bicollis in *Lepus cuniculus* to the single vagina and Uterus simplex in *Homo sapiens* (Fig. 3).

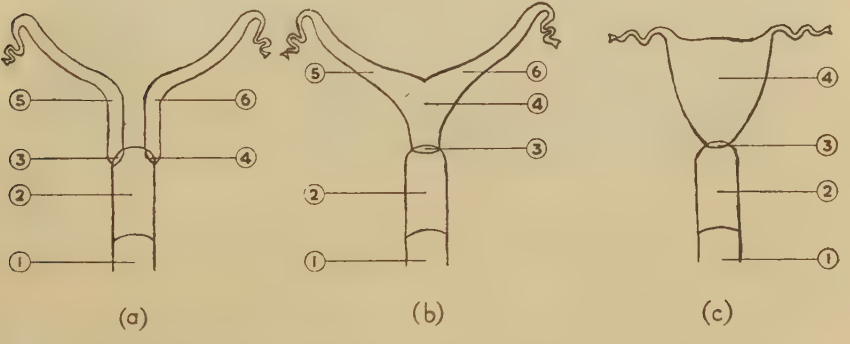

FIG. 3. Diagram showing the main stages in the evolution of the female genital tract among Eutherian mammals.

(a) *Uterus bicornis bicollis*, as seen in most of the Rodentia, *e.g.*, *Lepus cuniculus*· 1. Urogenital sinus. 2. Vagina. 3. Left cervix uteri. 4. Right cervix uteri. 5. Left uterus. 6. Right uterus. (b) *Uterus bicornis*, as seen in Carnivora, Proboscidea, Ungulata, Cetacea, Insectivora, and Prosimiæ, *e.g.*, *Bos taurus*. 1. Urogenital sinus. 2. Vagina. 3. Cervix uteri. 4. Corpus uteri. 5. Left cornu uteri. 6. Right cornu uteri. (c) *Uterus simplex*, as seen in Primates, *e.g.*, *Homo sapiens*. 1. Urogenital sinus. 2. Vagina. 3. Cervix uteri. 4. Corpus uteri.

Bissonnette (1924) studied the embryology of the genital tract of *Bos taurus* and stated that the Müllerian duct of each side begins to develop as a thickening of the coelomic epithelium of the 1·2 cm. fœtus about one quarter of the way caudal to the cephalic pole of the mesonephros and on the ventro-medial aspect of the corresponding Wolffian duct. In the 2·0 cm. fœtus the ostium abdominale tubæ is well formed and there is a lumen running some distance caudally in the duct. It is not, however, till the 3·0 cm. stage that the ducts have attained the level of the urogenital sinus. At the 3·8 cm. stage the ducts have become apposed to each other in the median plane at their caudal ends, but have not fused, and still terminate separately at the dorsal wall of the urogenital sinus. Fusion between the two ducts has begun to take place in the 5·1 cm. fœtus. There is fusion in the future vaginal and uterine regions with an intermediate section (presumably the future cervix) where the ducts remain separate. This point should be emphasised in view of the condition obtaining in the specimens described above. Bissonnette stated that in the 7·5 cm. fœtus the corpus uteri and vagina are single chambers with double channels between. He makes it quite clear that he examined the cervical region in the 15·0 cm. fœtus, but he unfortunately does not say whether or not duplicity was still present in that area.

On the Apparent Tendency in *Bos Taurus* to Duplicity of the Cervix Uteri.

In the course of his studies, Bissonnette found that, at about the 5·1 cm. stage, the cervical region of the genital tract in female, male, and freemartin embryos was double, while cranially and caudally there were single chambers. It would appear, therefore, that at the stage in question, this is the normal state in *Bos taurus*. Further, while in common with other species of placental mammals the right and left Mülleran ducts tend to fuse in the midline, there seems to be a delay in the region of the future cervix uteri, the ducts tending to remain separate for a longer period in that segment than in those segments which fuse cranially and caudally to it. This delay in fusion is present at least up till the 7·5 cm. stage, although normally complete fusion is probably to be found at the 15·0 cm. stage. This being so, it seems surprising that there are not more frequent records in scientific literature of what is probably a not uncommon abnormality in cattle.

According to Williams (1921) the condition is not at all rare in the ox, but only one record appears in the literature of the past ten years, *viz.*, in a paper by Stuhlenmiller (1933). Even in this record, although the author states that the whole length of the cervix was double, he does not say whether or not the corpus uteri was single ; his choice of words appears, however, to indicate that this was so.

Uterus bicornis bicollis has frequently been encountered in cattle, vide Craig (1937) and Stuhlenmiller (1933), and records may be found

PLATE II.

of Uterus bicornis bicollis combined with Vagina subsepta (Demeter, 1926); such conditions are also encountered in man where there is evidently not the delayed fusion of the Müllerian ducts in the cervical region. Such anomalies are examples of the persistence of the more primitive types of genital apparatus (compare the normal condition in *Lepus cuniculus* and in Marsupialia and Monotremata). Specimens such as those described above, on the other hand, appear to be the result of the innate tendency of the cervical region of the genital tract of *Bos taurus* to remain double, counteracting the more general tendency of the Müllerian ducts to fuse in mid-line and preventing such fusion taking place in that segment.

It is interesting to note that Macrae (1935) recorded a similar case of duplicity of the cervix in *Equus caballus*. The mare was examined alive, so that it was, of course, impossible to determine the exact state of the uterus. Macrae's description suggests that the condition was analogous to that described in *Bos taurus* in this paper.

No research appears to have been carried out on the embryological development of the genital tract either of *Equus caballus* or of any other species of the same genus. It would be interesting to know whether there is a delay in the unification of the cervical region similar to that which Bissonnette observed in *Bos taurus*.

Summary and Conclusions.

Three cases of Cervix bifida are described occurring in otherwise normal female genital tracts of *Bos taurus*. It is concluded that they are the result of persistence of the condition present in the 7·5 cm. fœtus which should normally have disappeared during subsequent fœtal life.

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References.

Baxter, J. S. (1935). Contributions to embryology. Vol. 25. Carnegie Inst. Wash. Pub. No. 459, pp. 15-35.

Bissonnette, T. H. (1924). *Amer. J. Anat.*, **33**, 267.

Craig, J. F. (1937). *Vet. Rec.*, **49**, 479.

Demeter, Hans. (1926). *Münch. Tierärztl. Wschr., Jahrbg.*, **77**, 514.

Macrae, D. R. (1935). *Vet. Rec.*, **15**, 1100.

Stuhlenmiller, M. (1933). *Tierärztl. Rdschr.*, **39**, 377-380.

Williams, W. L. (1921). Diseases of the Genital Organs of Domestic Animals. Andrus and Church, Ithaca, N.Y.

A NOTE ON THE OCCURRENCE OF *BR. ABORTUS* AGGLUTININS IN FARM HORSES IN THE EAST OF SCOTLAND

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Several workers in Europe and America have examined sera from apparently normal horses for the presence of agglutinins for *Brucella abortus,* and Priestley (1934) has conducted a similar survey in Great Britain. Of 264 army horses examined by him 39 per cent. were negative, 40 per cent. reacted at a dilution of 1/10, 16 per cent. at 1/20, and approximately 4 per cent. at 1/40 or more.

Great variation in the percentage of reactors has been reported by Continental workers. Thus in Germany, Fontaine and Lutje (1919) found 0·5 per cent. of 3,419 animals positive, Berg and Haupt (1937) 14·7 per cent. of 232 positive at 1/50, and Leskova (1937) examined 1,100 sera of which approximately 10 per cent. had a titre of 1/100 or more. In Holland, van der Hoeden (1930, 1932) found that of two groups of horses, over 900 in all, one showed 40 per cent. positive at 1/50 or more, and the other 36 per cent. In Albania, Herrmann (1934) examined 251 horses and found 17·9 per cent. positive at 1/100.

In the United States, Deem (1937) examined 103 artillery horses of which 9·7 per cent. reacted at a dilution of 1/50 or 1/100, Carpenter and Boak (1937) found 27 per cent. of 347 horses to react at 1/25, 14 per cent. at 1/50 and 8 per cent. at 1/100 or more. Stone (1938) tested the sera of 1,172 town horses of which 9·6 per cent. were positive at 1/50 and 205 country horses of which 23·9 per cent. reacted at the same dilution.

There is thus a wide divergence in the results obtained by workers in different parts of the world, which may perhaps be accounted for in part by variation in the antigens used, and in part by variation in the degree of exposure to natural infection.

In 1937, a number of equine sera became available in this Institute and were examined for agglutinins against *Br. abortus.* They were derived from apparently healthy farm horses in the counties of Aberdeen, Kincardine, Angus, Perth, Fife and East Lothian, in which general farming is commonly practised, and dairy farming as such does not predominate.

Technique.

The tube test described by Stableforth (1936*a*) in which final serum dilutions of 1/10, 1/20, 1/40, etc., are obtained, was employed, the only modification being the use of sterile normal saline in place of 0·5 per cent. carbol saline.

The antigen used was the standard abortus suspension prepared for the Agricultural Research Council (Great Britain) by the Royal Veterinary College, London, the preparation and standardisation of

which has already been described (Stableforth 1936*a*). The same author (1936*b*) has compared this antigen with those in use in other countries.

The end titre only was recorded, and was taken as the highest dilution of serum showing definite agglutination.

No intermediate zones (Seddon, 1915) were observed.

Results.

957 sera in all were examined, with the result shown in Table I:—

Table I.

Dilution.	*Number Positive.*	*Per cent.*
1/10	188	19·6
1/20	21	2·1
1/40	12	1·2
1/80	2	0·2
1/160	1	0·1
1/320	3	0·3
1/640	—	—
1/1280	1	0·1
Total positive at 1/10 or more	228	23·8
Total positive at 1/20 or more	40	4·1

The age and sex of all but 52 of the horses were known; it was therefore possible to determine the influence, if any, of age and sex upon the agglutination titre of the remaining 905 animals. Priestley (1934) has already shown that there is no significant difference between the sexes, but indicated that there might be a relationship between end titre and age. Leskova (1937) also stated that the number of positive animals increases with age. Their results were based upon an examination of 314 horses and 1,100 horses respectively.

In order to assess the influence of age and sex, the figures were arranged as in Table II:—

Table II.

Sex.	*Age in Years.*	0–2	3–4	5–6	7–8	9–10	over10
Male ...	Negative	57 (52)	139 (130)	120 (115)	61 (66)	32 (37)	32 (41)
	1/10	6 (11)	17 (27)	19 (24)	17 (14)	14 (7·7)	18 (8·4)
	1/20 and over	2 (2·6)	7 (6·5)	6 (5·8)	5 (3·3)	1 (1·9)	1 (2·0)
Female	Negative	44 (39)	93 (87)	47 (45)	35 (34)	17 (27)	14 (17)
	1/10	10 (13)	26 (29)	14 (15)	11 (11)	15 (9)	9 (5)
	1/20 and over	1 (2·4)	4 (5)	2 (2·8)	2 (2)	6 (1·7)	1 (1)

The figures in brackets are those calculated.

If the actual figures are compared with those calculated on the assumption that sex has no influence on agglutination titre, it is found that $x^2=21{\cdot}33$, $n=12$ and $P=0{\cdot}045$, which indicates that the influence of sex is of doubtful significance.

In determining the influence of age, the same test was applied to each sex separately, and in the male group $x^2=30{\cdot}01$, $n=10$ and $P<0{\cdot}0008$, while in the female group $x^2=25{\cdot}75$, $n=10$ and $P=0{\cdot}004$, both of which results are significant. It would appear, therefore, that age has a definite influence on end titre.

In determining the influence of sex, $P=0{\cdot}045$, which is barely significant, and as a reaction in a dilution of 1/10 is not universally accepted as positive in species other than equines, and as the titre which may be considered positive in horses has never been satisfactorily determined, the figures were rearranged as in Table III, in which the reactors at 1/10 have been combined with the negative group, and all reactors in a dilution of 1/20 or over have been arbitrarily considered positive.

Table III.

Sex.	*Age in Years.*	0–2	3–4	5–6	7–8	9–10	over 10
Male ...	Negative	63 (62·7)	156 (157)	139 (140)	78 (80)	46 (45)	50 (49)
	1/20 and over	2 (2·5)	7 (6·6)	6 (5·7)	5 (3·3)	1 (1·9)	1 (2)
Female	Negative	54 (51)	119 (117)	61 (60)	46 (45)	32 (36)	23 (22)
	1/20 and over	1 (2·5)	4 (5·6)	2 (2·8)	2 (2·2)	6 (2)	1 (1·1)

The figures in brackets are those calculated.

The x^2 test was again applied, with the result that following this arrangement sex is shown to have no significant effect on end titre as $x^2=6{\cdot}882$, $n=6$ and $P=0{\cdot}4$. The influence of age was again determined and found to have no significant bearing on end titre as in the male group $x^2=2{\cdot}119$, $n=5$ and $P=0{\cdot}8$, while in the female group $x^2=10{\cdot}327$, $n=5$ and $P=0{\cdot}05$.

Discussion.

It would appear that there is no significant evidence that sex has an influence upon the production of agglutinins for *Br. abortus* in horses, which confirms the observations of Priestley (1934).

With regard to age, there is evidence that the effect on the titres as recorded is significant, however, if the weak positives, *i.e.*, those reacting in a dilution of 1/10 only, be combined with the negatives this significance entirely disappears. This is confirmed by the close

agreement between expected and observed numbers in the 1/20 and over groups. The situation would seem to be that the older male animals exhibit a larger number of weak positives than would be expected. This is confirmed by an independent analysis of the two groups, negatives and positives at 1/10, which gives in the male group $x^2=27{\cdot}64$, $n=5$ and $P=0{\cdot}00004$, and in the female group $x^2=12{\cdot}58$, $n=5$ and $P=0{\cdot}05$. It is possible that this increase with age in the proportion of weakly positive sera may be due to non-specific agglutination or to the development of so called "natural agglutinins." In any case, the results give no support to the view that the number of frankly positive sera increases with age.

Summary.

The sera of 957 horses from agricultural districts in the East of Scotland have been examined by the tube test for agglutinins for *Br. abortus*.

769 gave a negative reaction to the test. Agglutinins were detected in 188 or 19·6 per cent. in a serum dilution of 1/10, in 21 or 2·1 per cent. in a serum dilution of 1/20, and in 19 or 1·9 per cent. in a serum dilution of 1/40 or more.

If those which showed the presence of agglutinins in a serum dilution of 1/10 are classed as negative, it would appear that neither sex nor age have a statistically significant influence upon the agglutination titre.

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References.

Berge, E., and Haupt, H. (1937). *Berl. tierärztl. Wschr.*, Feb. 12th, p. 97. (Abs. in *Vet. Bull.*, **7,** 518.)
Carpenter, C. M., and Boak, R. A. (1937). *J. Bact.*, **33,** 40.
Deem, A. W. (1937). *Ibid.*, **33,** 40.
Fontaine and Lutje. (1919). *Z. f. Veterinärkunde*, **31,** 1.
Herrmann. (1934). Quoted by Thomsen, A., *Bull. Off. Int. Epiz.*, **14,** 120.
Hoeden, van der. (1930). *Tidjschr. v. Diergeneesk.*, **57,** 15.
——— (1932). *Z. f. Haust.*, **42,** 1.
Leskova, R. (1937). *Wiener tierärztliche Monatsschrift*, **24,** 225-233. (Abs. in *Bull. Int. Off. Epiz.*, **15,** 325.)
Priestley, F. W. (1934). *J. Comp. Path. & Ther.*, **47,** 181.
Seddon, H. R. (1915). *Ibid.*, **28,** 20.
Stableforth, A. W. (1936*a*). *Ibid.*, **49,** 251.
——— (1936*b*). *Bull. Off. Int. Epiz.*, **12,** 107.

THE NATURE OF FOWL PARALYSIS (NEUROLYMPHOMATOSIS)

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There appears to be a general lack of agreement concerning the relationship of certain diseases of poultry and hence the literature is confusing and terms such as leucosis, lymphomatosis, leukæmia and lymphocytoma, are often used to describe either the same disease or a group of diseases believed to be allied. The disease under consideration is that described by Pappenheimer (1929) and by McGaughey and Downie (1930). It will be referred to throughout this article as fowl paralysis or neurolymphomatosis and means that condition in fowls in which there occurs abnormal accumulation of lymphocytes and certain other cells in the organs and nerves with, in some cases, actual tumour formation.

Despite the widespread occurrence of fowl paralysis and the attention it has attracted in almost all countries, its ætiology still remains obscure and opinions differ concerning its true nature.

Some workers hold the view that the occurrence of fowl paralysis is closely associated with the presence of parasites in the intestinal tract, the main evidence for their conclusions being the frequent association of the two conditions. Warrack and Dalling (1932) and others have shown clearly, however, that neurolymphomatosis can occur in the entire absence of parasites during the whole life of affected fowls.

The view has frequently been expressed that some dietetic deficiency may be the responsible agent. This has been disproved by several workers ; thus, Lerche and Fritzsche (1933) and Wilcke and his co-workers (1933) found no significant difference in the incidence of the disease when the rations contained varying quantities of minerals and vitamins. On the other hand Adamstone (1936) reported that vitamin E deficiency was largely responsible for the occurrence of a condition in fowls resembling leukæmia and Butler and Warren (1938) state that they have treated successfully field cases of fowl paralysis with wheat germ oil. Other workers (Jungherr, 1938, and Cole, 1938) as well as the writer (quoted by Jungherr, 1938) have failed to confirm their findings.

Although some workers are of the opinion that an infective agent is associated with the occurrence of fowl paralysis, definite proof is still lacking and other workers, *e.g.*, Oakley (1935), consider the condition to be neoplastic in nature.

Transmission through the egg is generally considered to take place, though some workers, *e.g.*, Asmundson and Biely (1932), are of the opinion that it is only a susceptibility which may be inherited. Furthermore, Dalling and Warrack (1936) have shown that the incidence of the disease varies widely in the progeny of related pullets and Lee and his co-workers (1937) are of the opinion that there is

a difference in the susceptibility of different strains and individual fowls to experimental infection.

Much experimental work on the transmission of fowl paralysis has been carried out, but the results in most cases must be regarded as inconclusive because of the occurrence of typical lesions in the control untreated fowls in the experiments. The usual method of transmission has been the injection of material from natural cases and, though successful results have been obtained, there remains some doubt concerning the specificity of the response, for Dalling and Warrack (1936) produced some evidence that typical lesions might be stimulated by the injection of normal fowl tissues.

In connection with experimental transmission work some workers have found various types of lesions in the injected fowls which were not present in the donors of the injected material, and consider this to be an atypical response. Thus, a confused picture of the disease has arisen. For example, Furth (1935) describes experiments in which fowls injected with material from a case of lymphomatosis developed myelomatosis and endothelioma which were transmissible and in which round cell accumulations occurred in the peripheral nerves. Likewise, the experiments carried out by Patterson (1936) suggest that erythroleucosis, and myeloid and lymphoid leucosis are all expressions of the same transmissible disease. He also concluded that the individual fowl and the strain are important factors in determining the type of disease.

Transmission by contact has been recorded but there is a lack of definite evidence on the subject. The tendency of the disease to affect only certain strains, especially when first observed on a poultry farm, has led to the belief that it is not contagious. Some workers, however, hold the opinion that spread by contact does occur and the recent observations of Johnston and Wilson (1937) support this view.

In previous work at this centre (Blakemore and Glover, 1934) neurolymphomatosis was transmitted to a limited number of fowls by the inoculation of emulsions of tissues from natural cases of the disease.

Further experiments in transmission have been carried out in which two strains of fowls were used, (*a*) an inbred strain from which fowl paralysis had disappeared and (*b*) a strain in which the disease had never been observed. Thus, a comparison of susceptibility of strains was possible.

Scope of the Experiments.

The experiments to be described can conveniently be grouped in two sections:—

1. The transmission of neurolymphomatosis to fowls from both sources by the inoculation of tissues from affected birds, and the fate of chickens when exposed to natural infection.

2. The passage of the infective agent through chickens and a study of the lesions produced.

The strains used in the Experiments.

The inconclusive results of many experiments have been due to the occurrence of fowl paralysis in the control, untreated groups, and it seems essential to secure for transmission work stock which is known to be free from the disease. The evidence regarding the passage of the infective agent through the egg also indicates that it is necessary to keep strains under observation for some considerable time before using them for experimental purposes. In the present investigation these points have received attention, and while the examinations were limited in one of the strains to two generations the other was kept under observation for a number of years.

The method of management was the same throughout the preparatory breeding work, the fowls being kept in outside pens which were changed when necessary. The ration was also constant. A mash composed of 41 per cent. middlings, 40 per cent. maize meal, 6½ per cent. bran and fish meal, 4 per cent. soya bean meal and 1½ per cent. dried milk, was given in the mornings. Whole wheat was supplied in the evenings and a liberal amount of fresh green food was allowed.

Strain P.—The foundation stock was hatched from eggs obtained from a cross-bred flock known to be severely affected with fowl paralysis. The fowls were kept in isolation and a high incidence of the disease occurred amongst them. In order to ascertain the effect of in-breeding upon the occurrence of neurolymphomatosis the fowls of this and each succeeding generation were inbred as pullets and on each occasion the progeny were reared in isolation. The fowls were autopsied at intervals and in all cases sections of parts of the nervous system were examined for the presence of cellular infiltrations characteristic of neurolymphomatosis.

The results (Table I) show that in the second generation fowl paralysis was present, though the clinical incidence as compared with that in the first generation was much reduced. In the third, fourth and fifth generations no cases were recorded and the general standard of health was high. Further, no other forms of leucosis were observed. This stock, therefore, had become free from fowl paralysis and formed an interesting strain on which to carry out transmission experiments.

Table I.

Generation.	*Number of Fowls.*	*Cases of Neurolymphomatosis.*			
		Clinical Cases.	*Gross Nerve Lesions.*	*Microscopical Lesions Only.*	*Total Incidence.*
1	35	7	7	11	18
2	31	4	2	12	14
3	19	0	0	0	0
4	36	0	0	0	0
5	59	0	0	0	0

Strain B.—The fowls from which the experimental chickens were derived were hatched from eggs obtained from a small flock in which clinical paralysis had not occurred. Very few fowls had been introduced during recent years and a certain amount of inbreeding had taken place. The strain had only been under observation for about two years, but the parents of the experimental chickens were hatched at the Institute and subjected to the same examinations as the P strain ; no cases of fowl paralysis were detected.

Part I. Transmission Experiments.

(a) The inoculation of chickens of both strains with tissues from natural cases of neurolymphomatosis.

The material for injection was taken from four young fowls obtained from one affected flock. The lesions on autopsy were characteristic of fowl paralysis and, with the exception of the donor of transmission 3, were restricted to the peripheral nerves. In this case lymphomatous tumours were also present in the liver and kidneys. Histological examinations revealed typical lymphocytic infiltrations in each instance and there was no evidence of any other disease. The tissues utilised were spleen, lung, liver, brain and peripheral nerves. They were removed, precautions being taken to avoid contamination, triturated with sand and suspended in saline. Time was allowed for the coarse matter to sediment and the supernatant was then used immediately.

In all, four transmission experiments were carried out. In each case the tissues from a single infected fowl were injected into a group of chicks from each strain.

The fowls were kept under the same conditions as in the preliminary breeding work and precautions were taken to prevent a spread of infection between the flocks.

Within a few days some of the chicks became ill and a number died. Bacteriological examinations failed to reveal any organisms to which these losses could be attributed, with the exception of transmission 2, in which *S. pullorum* was isolated. The inoculum in this case was found subsequently to be infected, and in view of the danger of spread of infection the survivors were killed, having been kept under observation for only a short time. The chicks which died from the infection were not subjected to further study and thus the number of subjects in transmission 2 was much reduced.

After the few early deaths, the mortality was low for a time but the injected chicks had a general unthrifty appearance when compared with the controls.

Clinical symptoms of paralysis were first seen in a chicken in transmission 2 of the P group, 38 days following inoculation, and a number of marked clinical cases were observed shortly afterwards. Within 88 days after inoculation, eight paralysed chickens had been autopsied and others were reserved for observation (Fig. 1). The occurrence of paralysis was delayed in the B group for, although one

fowl had lesions when killed 85 days after injection, the majority of the cases occurred later. The total incidence of fowl paralysis in the two groups is shown in Table II and it will be observed that little difference was found, though the shorter period of incubation in the P strain suggests a higher degree of susceptibility of that strain.

TABLE II.

THE INCIDENCE OF NEUROLYMPHOMATOSIS IN THE VARIOUS GROUPS AS DETERMINED BY THE PRESENCE OF NERVE LESIONS.

1. *P. Strain.*

	Inoculated.		*Controls.*		
	Number of Fowls.	*Number Affected.*	*Number of Fowls.*	*Number Examined.*	*Number Affected.*
Transmission 1	18	9	30	12	0
,, 2	6	1			
,, 3	29	11	33	21	0
,, 4	25	2	30	13	0
Total	78	23	93	46	0

2. *B. Strain.*

	Number of Fowls.	*Number Affected.*	*Number of Fowls.*	*Number Examined.*	*Number Affected.*
Transmission 1	7	3	13	13	0
,, 2	3	0			
,, 3	35	18	16	16	1
,, 4	12	1	13	13	0
Total	57	22	42	42	1

The unthriftiness which was apparent shortly after inoculation persisted for some considerable time and is well illustrated in Table III, in which the average weights of all groups are shown approximately four months later. The injected fowls showed a disinclination to move about, often standing for long periods in one corner of the run. Depigmentation of the iris was observed in some of the fowls, and in a few lesions of iritis were present with consequent constriction of the pupil and impaired vision.

TABLE III.

	Controls.						*Experimental.*					
	Males.			Females.			Males.			Females.		
	No.	lbs.	ozs.	No.	lbs.	ozs.	No.	lbs.	ozs.	No.	lbs.	ozs.
P. Trans. 1 ...	11	5	15	14	4	7	4	4	10½	10	3	7
P. Trans. 3 ...	10	3	13	18	3	4	13	3	10	10	2	11
P. Trans. 4 ...	5	2	11	9	2	3	5	2	6	7	1	13
B. Trans. 1 ...	5	5	9	7	4	14	1	5	1	1	2	10
B. Trans. 3 ...	6	4	12	9	3	11	13	3	15	20	2	15
B. Trans. 4 ...	6	4	6	5	3	10	3	2	10	6	2	4

To exclude the possibility that the above symptoms in the non-paralysed fowls arose as the result of some extrinsic factor, bacteriological examinations were made of a number of typical affected specimens and a significant percentage of the stock was subjected to agglutination tests for the detection of Salmonella and Listerella infections. The results obtained were entirely negative.

Observations were also made on the content of the intestines of the slaughtered fowls, but no evidence was abduced that the condition was due to the effect of parasites. Indeed, the flocks remained particularly free from parasites for the first four months, although later lesions attributed to *E. necatrix* were occasionally found and fairly heavy infestations with other parasites were sometimes also detected. The late association of parasites with the experimentally produced condition is of particular interest since it appeared as though the general unhealthy state of the stock may have served as a predisposing cause.

In the later stages of the experiment some general improvement in the fowls was noticed and no gross changes were found in those which were killed at that time. The number of survivors was, however, small, and it was decided to kill all of them and terminate the experiment.

(*b*) *Post-mortem findings.*

The practice adopted was to allow symptoms to become developed before the fowls were slaughtered, since it was thought that this would facilitate the detection of lesions in the nerves. The times after inoculation when experimental fowls were examined are shown in Fig. 1. With the exception of the few fowls killed in the final stages of the experiment, all either died or were slaughtered after showing evidence of paralysis or unthriftiness. The results in both strains and all transmission groups were identical and will be discussed together.

In the non-paralysed fowls the *post-mortem* findings varied according to the period after inoculation when they were killed. The majority of the fowls killed within 150 days of injection showed gross lesions, especially in the heart and liver, although slight changes of a similar nature were often observed in some of the other organs. The pericardial sac usually contained a small quantity of clear serous fluid and whitish ill-defined areas were visible in the myocardium. In cases in which these changes were not pronounced the heart had a characteristic flabby appearance without any other apparent abnormality. The liver lesions varied enormously. In some cases the organ was slightly enlarged and covered with a layer of gelatinous material, but in other instances it was of normal size and the capsule was unchanged. Whitish areas of varying size were frequently present in its substance. In many of the specimens these lesions were small and evenly distributed throughout the organ, but in others extensive areas of the liver were destroyed. Even when the changes

were advanced there was no tendency towards encapsulation or diffuse cirrhosis. Similar changes were also occasionally observed in the lungs, spleen and kidneys, but to a less marked degree. Swelling of the spleen was rarely encountered.

In the majority of the paralysed fowls there was a general loss of condition and the organs showed the above changes ; myocardial lesions were frequently seen and whitish areas were often present in the liver. Occasionally, however, even in early cases, no such visceral abnormalities were found. In most of the birds killed from five months after inoculation to the end of the experiment the picture presented was more consistent with typical neurolymphomatosis. Usually the only additional lesions were tumour formations or a slight flabbiness of the heart muscle, which could easily have been overlooked.

Gross nerve lesions were found in practically all the fowls which had shown paralytic symptoms. The changes were identical to those found in the natural disease and call for no special comment. In the majority, only the nerves supplying the limbs were affected, but in others the sympathetic, vagus and intercostal nerves were also involved.

The occurrence of lymphomatous tumours is of particular interest in view of the fact that they are considered by most observers to be a manifestation of fowl paralysis. The affected fowls are indicated in Fig. 1. Taking into account all groups, lymphomata were found in 8 per cent. of the cases, which is considerably lower than the figure of 46 per cent. given by Dalling and Warrack (1936) for the series of 128 cases of fowl paralysis which they examined. Reference to Fig. 1 reveals that the development of tumours occurred relatively late in the experiment, since no affected fowls were autopsied before the ninetieth day after inoculation. It would appear, therefore, that they represented a terminal effect of the stimulus and it may be assumed that the percentage of positive cases would have been greater if a higher proportion of the stock had survived to the susceptible age.

Sections of the nerves of each experimental fowl were prepared and in the majority of cases the organs were examined. The histological appearance was essentially the same as was observed in the chickens used in the subsequent passage experiments and will be considered in the next section.

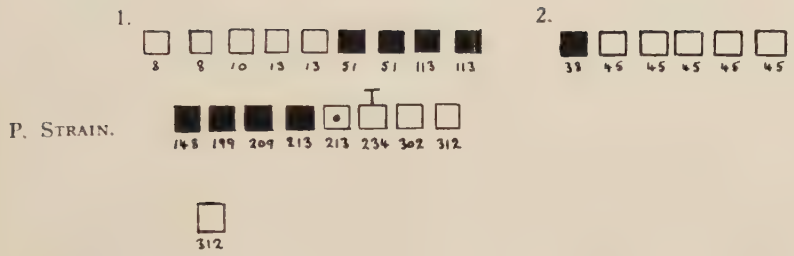
(c) The control fowls.

The number of control fowls of the P strain autopsied is shown in Table 2. The fact that no symptoms were observed in other fowls of the same generation, reserved for breeding, indicates further the freedom of the stock from fowl paralysis.

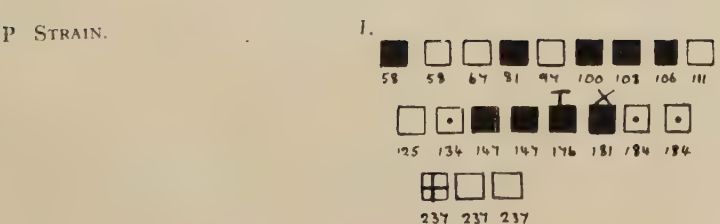
The B strain presented an interesting problem, as one quite definite case of fowl paralysis occurred in the controls of transmission 3. The development of neurolymphomatosis in a strain which was

FIG. 1. THE TRANSMISSION OF

INOCULATED G



GROUPS EXPOSED TO THE NATURAL



The numerals indicate days after injection or exposu

believed to be free is of importance. There was, however, definite evidence that the isolation of this group had not been satisfactory since, for a time, it was penned in close proximity to a group of fowls in which the disease occurred and it seems probable that a spread of infection took place.

The control fowls were killed at various times during the experimental period in order to ensure that no abnormality was overlooked at any stage.

(*d*) *Exposure of chickens of the P strain to natural infection.*

Advantage was taken of the apparent freedom of the P strain from fowl paralysis to obtain some information regarding the fate of the stock when reared in contact with natural cases of the disease. In this connection two small scale experiments were carried out in which day-old chicks hatched at the Institute were reared on infected premises. These experiments were carried out concurrently with the transmission work and were controlled by stock kept at the Institute as well as by a group of chickens which were dispatched to a smallholding where the disease had not occurred.

Experiment 1.—The owner had experienced a high mortality in his flock for a number of years and it was from him that the P strain was originally obtained. The chicks he received were reared on infected land together with a batch of his own chicks, which later proved to be severely affected.

Some unthriftiness was soon apparent in the exposed group and examinations revealed that they had become severely infested with coccidia and other parasites. Fowl paralysis became evident, the first fowl showing symptoms 58 days after exposure. From then onwards the incidence of the disease was high and by the 184th day 17 of the fowls had been autopsied and the remaining three were returned for observation. The three fowls improved in condition, but one of them developed paralysis later and all three were killed after an experimental period of 237 days.

Information regarding the individual fowls is shown in Fig. 1. The percentage of positive cases was very high and it was apparent that the high standard of health of the P strain when kept in isolation was not due to any inherited resistance to lymphomatosis.

The exposure of the P strain to natural infection produced much the same effect upon the general health as did the injection of tissues from natural cases of the disease. This may have been due to parasitism but the possibility of some other factor (the infective agent of fowl paralysis) cannot be altogether excluded. The *post-mortem* examinations did not show such striking lesions in the viscera as in the injected stock and the picture was more consistent with recorded descriptions of fowl paralysis. Occasionally, however, slight changes were found in the heart and the cellular response in the viscera was often of a very mixed kind.

Experiment 2.—In this instance 13 chickens were kept in contact

with a small flock in which cases of fowl paralysis occurred before and during the period of the experiment. The chickens were brooded by a hen and reared under natural conditions. The results (Fig. 1) show that the disease appeared in only two of the fowls, but here again the losses were high, for seven were returned for examination during the experimental period. Moreover, the general condition of the stock was similar to that in the previous experiment and the *post-mortem* findings were identical. There was not the same degree of chronic parasitism, however, although one case of acute cæcal coccidiosis occurred.

Experiment 3.—This experiment served as a control on the preceding work, since the chickens were reared on a smallholding where the disease had not occurred and it also provided some information regarding the behaviour of the P strain when not maintained in isolation. Thirty-four day-old chicks were transferred when a few days old and kept in contact with other stock for 280 days. For convenience the cockerels were slaughtered when 4-6 months old, so that only 18 pullets were under observation for the entire period of the experiment. Unfortunately it was not possible to obtain the fowls for post-mortem examination, but it is significant that the group was reared without loss and remained normal throughout.

Discussion.

By inbreeding a flock of fowls affected with neurolymphomatosis the disease was eliminated and following the disappearance of paralysis there was also no further occurrence of visceral lymphomatosis in the strain. Moreover, the general health was much improved and a negligible mortality rate was then experienced. That the strain was still highly susceptible is shown by the occurrence of the disease when chicks were exposed to natural infection. On the other hand, chickens reared in contact with a flock in which fowl paralysis did not occur remained healthy. The results of the experiment were so striking that there seems no reason to doubt further the infectivity of the disease. It also follows that the policy of many farmers in purchasing strains in which there is no history of fowl paralysis affords no safeguard against the disease, for it may simply mean that the fowls have not been exposed to infection.

Successful transmission of the infective agent was achieved by inoculating chickens of both strains with material from natural cases and, as the period of inoculation was shorter in the P group, it would seem that they were more susceptible. The inoculation had a striking effect upon the experimental chickens, which could not be accounted for as the result of any intercurrent infection. This effect was not inconsistent with the picture presented in many natural outbreaks in which a large number of the fowls become unthrifty without the occurrence of paralytic symptoms. A high mortality, usually regarded as being due to other causes, is also recorded in many published accounts of transmission experiments. For

example, Warrack and Dalling (1936) experienced a high total death rate, which was not attributed to fowl paralysis in the groups of fowls they injected. It might be pointed out, however, that in some flocks occasional cases of fowl paralysis do occur without any evidence of unthriftiness. The possibility that the atypical response may have been the result of the injection of two agents will be considered in the second section of this paper. It must be stressed, however, that the donors were obtained from one farm, that each showed typical lesions of neurolymphomatosis, and that similar results were obtained in the four separate experiments.

The high incidence of neurolymphomatosis in the inoculated groups is somewhat surprising in view of the failure of most workers to obtain such a high percentage of cases by a similar procedure. The explanation of this result must wait further study, but it seems possible that it may be due to the high susceptibility of the strains employed. It is also worthy of note that some variation in the incidence of the disease was recorded in the different groups and in P, transmission 4, the number of cases was comparatively low, the figures being in accordance with many published accounts of work of this kind.

Part II. The Passage of the Agent and a Study of the Lesions.

While it was assumed from the foregoing results that neurolymphomatosis had been set up in the experimental groups as a direct result of the inoculations, the high percentage of cases with heart and liver lesions was rather disconcerting. Experiments were, therefore, undertaken at an early stage in the work in order to ascertain whether this atypical condition could be transmitted by serial passage to young chickens. For this purpose the injection material was selected from a fowl which showed no evidence of nerve involvement but only the visceral type of lesion already described.

The first series of chicks to be injected was obtained from the B strain and in the subsequent experiments the chicks from the P strain were used. The chicks were 7-14 days old when injected. The method of management was also similar, but in passages 4 and 5 the isolation was improved by keeping the chicks indoors throughout the whole experimental period. The earlier passage groups were fed on a standard ration which is widely used in the industry and a supplement of cod-liver oil was given. Wheat germ oil was added to the ration of the chicks in passage 3. In the later experiments the diet was modified and contained the ingredients used for the stock in the previous transmission work. It was not possible to provide controls for each experiment, but an equal number of inoculated chickens were reserved in passages 4 and 5 and they remained normal.

There seemed to be no immediate effect of inoculation upon the first series of chicks and an examination of two carried out after

15 days revealed no macroscopic alteration in their organs. Shortly afterwards, however, a further two subjects were noticed to be sick and on *post-mortem* examination some slight abnormality was observed in the liver of one of them. A liver emulsion from both was injected into another series of chicks and this procedure was repeated through five groups, material from an acute case in the previous passage group being used in each case (Fig. 2). After the second series the effects of the inoculations became more apparent and acute symptoms were then observed approximately seven days later. The condition of the chicks was poor, the feathers were constantly ruffled and there was marked disinclination to move about. The virulence of the agent continued to rise and in the fourth passage nine of the eleven injected chicks had died within 32 days. Rapid passage was discontinued after the fifth experiment owing to the difficulty of obtaining a supply of suitable chickens.

The survivors of the acute phase were kept under observation for a considerable period. They remained unthrifty and a number of them developed symptoms of paralysis. In this connection the findings of the experiments were, therefore, in entire agreement with the original transmission work.

The intraperitoneal route was used almost exclusively, but in passage 4 two chickens received intravenous injections, three were injected subcutaneously and three intravenously. The results were comparable to those previously recorded, but it is interesting to note that the period of incubation was longer when the material was injected by the subcutaneous and muscular routes. In passage 5 a small number of chicks was inoculated with laked blood. Again the result was positive but the process was delayed.

The lesions in the acute disease following passage of the agent were more marked than those recorded in the case of the transmission experiments. The most pronounced changes were found in the heart, which was enlarged and presented a striking appearance when the thorax was exposed. Whitish areas were dispersed throughout the organ and, owing to their presence, the myocardium had a smooth but irregular surface. Occasionally the pericardial cavity contained a clear gelatinous layer. The liver was also invariably affected and lesions similar to those seen in the heart were found in its substance. Occasionally, they appeared as small foci scattered throughout the organ (Fig. 3) but in other specimens there was widespread destruction of the tissue. The spleen, kidneys, and lungs were also sometimes simlarly affected, but rarely to the same extent.

The survivors of the acute disease did not show such marked lesions on autopsy and the changes were identical with those already described in the original transmission fowls. The nerve changes in the fowls which developed neurolymphomatosis were mainly gross, and there was usually, but not invariably, some visceral disturbance.

Lymphomatous tumours were found in only one of the injected chicks, several nodular growths being observed in the liver 188 days

after injection. This finding is further evidence that the lymphomatous growths represented a late effect of the infective agent.

Nerve lesions were not found in any of the fowls till at least 55 days after the original injection. This finding is again in agreement with the results of the earlier experiments. The rapid passage of the agent did not increase the incidence of such cases. In fact, it would seem that the rise in virulence tended to decrease its action upon nervous tissues, as judged by the accumulation of cells of the lymphatic type between the nerve fibres, for although an occasional case occurred during the acute phase, the majority which became affected could be classed as "survivors." The same type of reaction was found in the organs no matter which route of injection was employed and local lesions did not occur when the injections were made subcutaneously or intramuscularly. Because of the small number of chickens in each group no conclusions could be formed regarding the method of injection most favourable to the stimulation of nerve lesions, but, generally speaking, the occurrence was dependent upon the duration of the disease.

Histological Findings.

The nerves of all of the fowls in both the original transmission work and the passage experiments were examined, and in most cases a study was also made of the viscera. As indicated by the *post-mortem* findings the only difference between the tissue changes in the transmission and passage groups was one of degree and they can be dealt with together. Moreover, a consideration in this way enables a more complete description of the disease to be given since it was only in the passage chickens that the acute phase could be properly studied.

The tissues reserved for further study were fixed in 10 per cent. formol saline and paraffin sections were prepared later. Hæmatoxylin and eosin were employed as the routine stains, although on occasion Van Gieson's method was used. In a few selected cases frozen sections were stained by Scharlach R for demonstration of fat.

When the internal organs were affected the main changes were found in the liver and heart and the process was essentially similar. The first change to be detected was destruction of the cells, but this was only clearly demonstrated in the passage chickens. In the liver the cells lost their affinity for strains, and, while the areas were usually focal, widespread necrosis was observed in some instances. In frozen sections it was apparent that the process commenced as a fatty degeneration. This necrotic lesion is shown in Figs. 4 and 5, which illustrate a typical reaction ten days after inoculation with passage material.

Later, these areas were found to be infiltrated with cells of various types. Lymphocytes were abundant (Fig. 6); large mononuclear and plasma cells were commonly found and many eosinophilic polymorphonuclear leucocytes were frequently present.

Fibroblasts were also found especially between the cardiac fibres and the reaction was frankly inflammatory in character. In some of the very acute lesions encountered during the course of the later passage experiments, hæmorrhage into these parts was apparent. In many cases the affected areas were densely packed with cells of the above-mentioned types and these cellular collections could be seen proliferating into the surrounding tissue. Later, however, they formed well defined centres and the limiting cells were usually large with abundant cytoplasm possessing nuclei with a well marked chromatin structure. These collections were mainly around the small hepatic vessels, although in some instances the portal tracts were involved to a considerable extent.

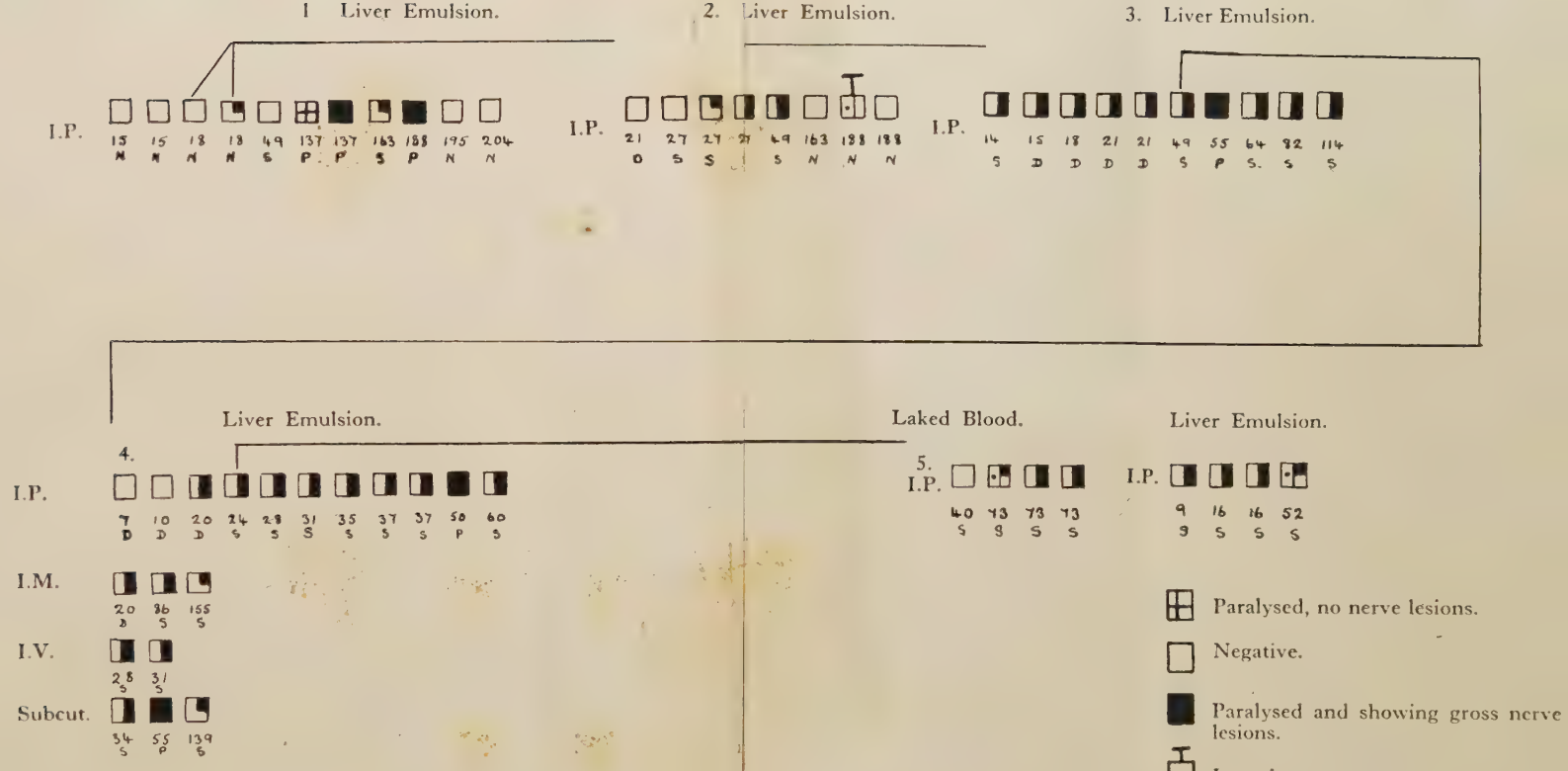
The picture was sometimes complicated by the presence of proliferating fine fibrillary tissue. In such cases the condition resembled lymphoblastomatosis described by Adamstone (1936), who considered that the proliferating tissue was derived from the endothelial walls of the sinusoids in the case of the liver and from the loose connective tissue between the muscle fibres of the heart and gizzard when those organs were affected. Nerve lesions were not recorded in his experiment and he regarded vitamin E deficiency as being an essential factor in the development of the condition he described. This resemblance to Adamstone's condition is more interesting in view of the fact that the gross lesions in the passage chickens were also similar. Early in the work the possibility that vitamin E deficiency may have influenced the results was considered and a liberal amount of wheat germ oil was added to the ration of passage 3. This supplement had no effect on the course of the disease.

The true lymphomatous response was seen most clearly in fowls killed from three months onwards following the injections, but even then only in a percentage of cases were typical lymphomatous centres found in the organs. When present (Fig. 7) they appeared as neoplastic areas without any well marked reaction of the surrounding tissue. All degrees of mixed lesions were, however, observed and it was often difficult to classify the nature of the response. When the true lymphomatous lesions attained a considerable size they had been recorded as tumorous masses on autopsy, and though usually diffuse in character they occasionally appeared as nodular growths.

Significant changes of some kind were found in the organs of the majority of the injected fowls, but it is interesting to note that a number of cases of neurolymphomatosis were unaccompanied by changes in the organs. Some of the passage chickens were good examples, *e.g.*, chick 7, passage 3, which was killed at a time when gross changes were being detected in the organs of other members of the same group.

The appearance of the nerve lesions in the positive cases calls for no special comment, for the cellular infiltrations were usually pronounced and presented the usual characters. When the nerves were

FIG. 2. PASSAGE EXPERIMENTS.



The numerals indicate days after injection or exposure when fowls were examined.

only slightly enlarged the infiltrating cells were mainly lymphocytes, but in old standing cases the cells had decreased in number and were replaced, in some cases almost entirely, by larger cells closely resembling mammalian plasma cells. This transition in the nerve lesions is well seen in natural cases of the disease and has been adequately described by other workers.

As in previous experiments (Blakemore and Glover, 1934) cellular accumulations which were regarded as diagnostic were observed in a proportion of the macroscopically normal nerves.

Discussion.

In the experiments recorded in the first section of this paper attention was drawn to an atypical kind of response which occurred in chicks injected with material from typical cases of neurolymphomatosis. Batches of chicks were injected in series with emulsions of the liver of infected chicks, and in all the disease was passaged in five groups. The passage increased the virulence of the agent so that the disease assumed a more acute character and thus a closer study of its nature was possible. The type of lesion in all cases remained essentially the same, but in the very acute disease the process was more truly inflammatory in character. In some of the survivors of the acute phase lesions of true lymphomatosis were found.

The occurrence of the atypical lesions and true neurolymphomatosis in the same fowls or in fowls of the same injected group is of considerable importance in considering the nature of fowl paralysis. In the above experiments it is conceivable that two entirely different infective agents have been passaged in the injection material, one giving rise to the visceral lesions found in the more acute stages of the disease and the other producing a lymphomatous response as a direct effect of the agent. From a study of the lesions in all stages of their development, however, it seems more likely that one agent only was present in the injected material. The initial effect of the agent is probably represented by the early lesions in the acute disease, inflammatory in character; and the true lymphomatous response, found in typical fowl paralysis, may represent a later, more chronic stage of the infection. Though it may be possible to stimulate lymphomatosis by different methods, the findings of this investigation are in keeping with field observations in which actual paralysis is of low incidence but is often accompanied by a general unthriftiness and mortality among growing stocks of fowls.

While it is not proposed in this paper to discuss the many published accounts of the pathology of neurolymphomatosis, it must be pointed out that although opinion is fairly unanimous that the iritis which often accompanies the disease is predominantly an inflammatory condition (McGaughey and Downie, 1930; Findlay and Wright, 1933), only a few workers express the opinion that the other

lesions start in this way. It is, however, interesting that Pappenheimer *et al.* (1929) who made a study of the pathology of the disease record a case in which a large part of the myocardial tissue of the ventricle was replaced by granulomatous areas containing a variety of cell forms, including fibroblasts, giant cells and lymphocytes, often surrounding remains of necrotic fibres. They also comment upon the undoubted transition between lesions which appear to be inflammatory and those which assume the character of a true neoplasm.

No detailed study has yet been made of the agent causing the changes. It is interesting to note, however, that Lee and Wilcke (1939), who consider that erythroid, myeloid and lymphoid lesions may occur following the injection of material from cases of fowl paralysis, have produced evidence that the casual agent can be filtered and behaves like a virus when subjected to electrophoresis. Further work on the nature of the agent is in progress.

General Summary.

By inbreeding, fowl paralysis (neurolymphomatosis) was eliminated from a strain of fowls derived from badly affected stock.

The progeny were still highly susceptible as shown by the occurrence of the disease following injection of tissues from natural cases and exposure to natural infection.

In addition to the occurrence of typical lesions of neurolymphomatosis the injected and exposed fowls became unthrifty and developed atypical lesions in the viscera, especially the liver and heart.

Passage experiments from the atypically affected fowls were carried out in the course of which the infective agent became raised in virulence and gave rise to an acute disease.

The early lesions in the heart and liver were inflammatory in character and in some of the survivors a true lymphomatous response was found.

A study of the lesions in all stages of the infection indicates that in all probability the disease, popularly termed fowl paralysis, or neurolymphomatosis, is the chronic stage of an acute infection.

The nature of the infecting agent is now being studied. The results of the investigation are in keeping with observations made in naturally occurring outbreaks of fowl paralysis and leave little doubt concerning the infective nature of the disease.

Acknowledgment.

The above investigation was made possible by a grant given by the Agricultural Research Council. The writer gladly welcomes the opportunity of expressing his thanks to Professor Dalling for his generous assistance and to Mr. F. D. Asplin, who helped in carrying out the experiments during his stay in Cambridge.

FIG. 3.—Organs of a chick showing early lesions of the acute disease in the liver.

FIG. 4.—Passage chick. Section of liver showing focal necrosis, 10 days after inoculation. Haematoxylin and eosin. × 70.

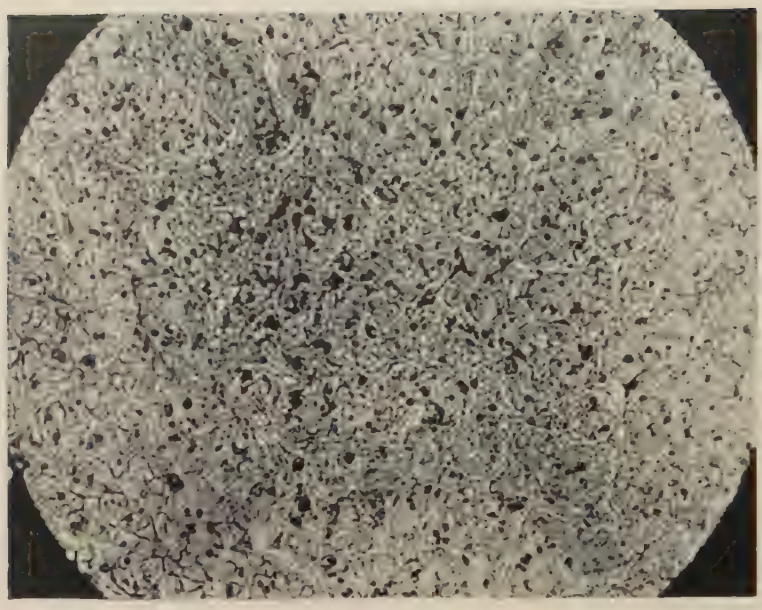


FIG. 5.—Fatty degeneration in the liver of an acute case of the disease. Sharlach R. × 400.

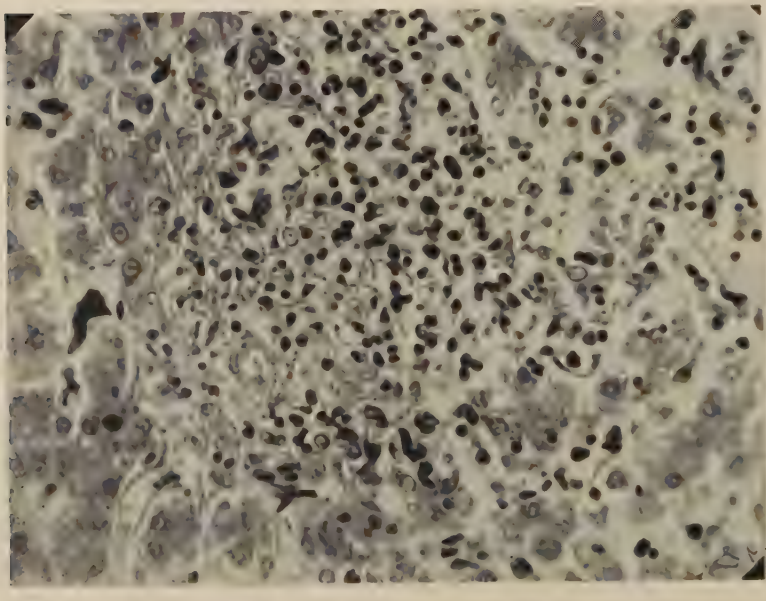


FIG. 6.—Section of liver, 15 days after inoculation, with passage material showing early lymphocytic infiltration into a necrosed area. Haematoxylin and eosin. × 400.

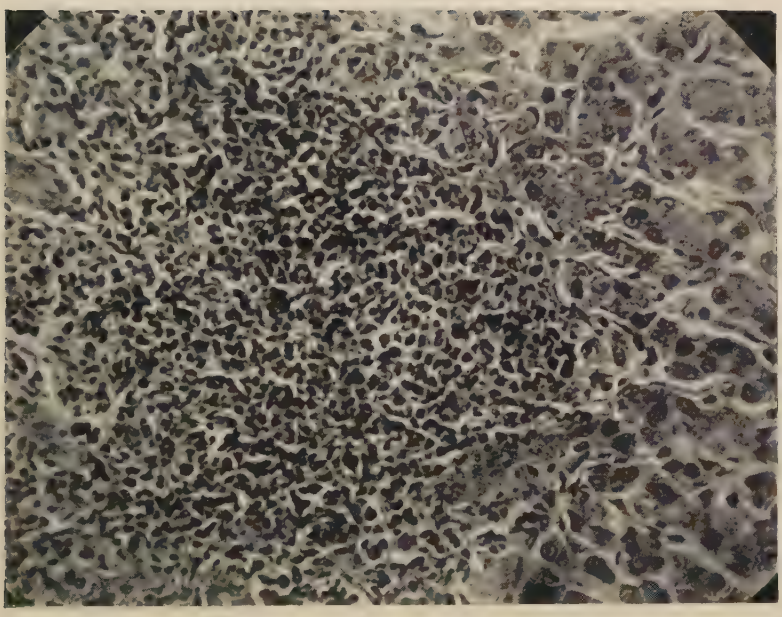


FIG. 7.—Section of liver 5 months after inoculation showing a well-defined lymphomatous centre. Haematoxylin and eosin. × 400.

References.

Adamstone, F. B. (1936). *Amer. J. Cancer*, **28,** 540.
Asmundson, K. S., and Biely, J. (1932). *Canad. J. Res.*, **6,** 171.
Blakemore, F., and Glover, R. E. (1934). *Rept. Inst. Anim. Path.*, **4,** 51.
Cole, R. K. (1938). *Science*, **88,** 286.
Dalling, T., and Warrack, G. H. (1936). *Vet. J.*, **92,** 310.
Findlay, G. M., and Wright, J. (1933). *J. Comp. Path.*, **46,** 139.
Furth, J. (1934). *J. Exp. Med.*, **59,** 507.
Johnston, J. W., and Wilson, J. E. (1937). *Vet. J.*, **93,** 13.
Jungherr, E. (1938). Proc. 8th An. Conf. Northeast, Proc. U.S. Live Stock Sanit. Assn.
Lee, C. D., and Wilcke, H. L. (1939). *J. Amer. Vet. Med. Ass.*, **46,** 178.
Lee, C. D., Wilcke, H. L., Murray, C., and Henderson, F. W. (1937). *J. Amer. Vet. Med. Ass.*, **44,** 147.
Lerche and Fritzsche, K. (1933). *Z. Infektkr. Haust.*, **44,** 11.
Oakley, C. L. (1935). *Proc. Roy. Soc. Med.*, **28,** 999.
McGaughey, C. A., and Downie, A. W. (1930). *J. Comp. Path.*, **43,** 63.
Pappenheimer, A. M., Dunn, L. C., and Cone, V. (1929). *J. Exp. Med.*, **49,** 63.
Patterson, F. D. (1936). *J. Amer. Vet. Med. Ass.*, **41,** 33.
Warrach, G. H., and Dalling, T. (1932). *Vet. J.*, **88,** 28.
Wilcke, H. L., Patterson, F. D., Henderson, E. W., and Murray, C. (1933). *Poultry Sci.*, **12,** 226.

THE OCCURRENCE AND PREVALENCE OF GASTRO-INTESTINAL HELMINTHS IN APPARENTLY HEALTHY CATTLE IN QUEENSLAND, AUSTRALIA

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A correct diagnosis of disease associated with parasitic infection is not always a simple procedure, particularly in the case of parasitic gastro-enteritis, where the symptoms may be simulated by those of other disease entities. A *post-mortem* is undoubtedly of value in assisting an opinion, but we know very little of the pathogenicity of the various helminth species and of the numbers of these species which must be present to cause disease. Such a knowledge is best gained by establishing infections of single species in worm-free animals, observing the effects of the infections and later on determining the numbers present on *post-mortem*. Alternatively, we can examine a sufficiently large series of healthy animals, count or estimate their helminth populations and thus secure some idea of the numbers in which a species must be present to be regarded as pathogenic.

Parasitic gastro-enteritis is at certain periods of the year a very common disease of cattle in Queensland. In making an investigation into the etiology and control of this disease, preliminary work was undertaken to ascertain what species of helminths were present in the alimentary tract, their prevalence and the populations that existed in healthy animals. Attention was confined to calves and yearlings, as it is among such young animals that outbreaks are most common. Every animal examined had been slaughtered for human consumption and was therefore in a presumably healthy condition.

A total of 237 animals was examined. Of these only 2·87 per cent. were free of infection. From the remainder, 29 species of helminths were collected and are discussed in detail below.

Trematoda.

Genus Paramphistomum Fischoeder, 1901.

P. cervi (*Schrank,* 1790) *and P. explanatum* (*Creplin,* 1847).

These two species are considered together. Both occur in the rumen and reticulum and are at times very difficult to separate. These flukes are exceedingly common among grown cattle in the coastal areas and heavy infections in apparently healthy animals are by no means infrequent. In this survey, 12·7 per cent. of the animals were infected, the largest number of flukes present in any one animal being 326. Numbers of immature forms were found on several occasions in the abomasum, duodenum and anterior jejunum.

Genus Cotylophoron Stiles & Goldberger, 1910.

C. cotylophorum (*Fischoeder,* 1901).

This species was encountered on four occasions, 6, 8, 12 and 32 specimens respectively being present.

CESTODA.

Genus Moniezia R. Blanchard, 1891.

M. expansa (*Rudolphi,* 1810).

Apparently a rare species in cattle in this part of the world, as it was seen only twice, each infection consisting of a single specimen.

M. benedeni (*Moniez,* 1879).

This is the common cestode of Queensland cattle. It was present in 11·7 per cent. of the animals. A six months calf yielded the largest number of specimens, namely, ten.

Genus Helictometra Baer, 1927.

H. giardi (*Moniez,* 1879).

It is inteersting to note that *H. giardi,* which is the common tapeworm of cattle in South Africa, has been seen only once in cattle in Queensland.

NEMATODA.

Genus Hæmonchus Cobb, 1898.

H. contortus (*Rudolphi,* 1803).

This was the most frequent species encountered and was present to the extent of 86·7 per cent. In the youngest animals (2 to 4 months) the majority yielded less than 100 worms each, but two animals had infections of 846 and 920 respectively. In the next age group 5 to 8 months), 66·2 per cent. had light infections, *i.e.,* less than 100 worms each, 26·7 per cent. between 100 and 1,000 worms, and in 7·1 per cent. the infections varied from 1,000 to 2,200 worms. In the oldest group (9 to 15 months) 20·4 per cent. contained 100 worms or less, 60·3 per cent. between 100 and 1,000 worms and 19·3 per cent. from 1,000 to 2,500 worms respectively. The highest number of worms to be taken from any animal was 2,500, from a 15 months old heifer.

Genus Ostertagia Ransom, 1907.

Three species of this genus were recorded, namely, *O. ostertagi* (Stiles, 1892), *O. circumcincta* (Stadelman, 1894) and *O. occidentalis* Ransom, 1907.

O. ostertagi (*Stiles,* 1892).

This species was found in 75·2 per cent. of the animals examined. The majority of these, namely, 72·8 per cent., yielded less than 1,000 worms each, 25 per cent. from 1,000 to 5,000 worms and 2·2 per cent.

from 5,000 to 7,500 worms respectively. The largest number seen was 7,500 worms which were taken from a 12 months old animal.

O. circumcincta (*Stadelman,* 1894).

Fifty specimens of this species were collected from one animal.

O. occidentalis Ransom, 1907.

This species was present in 4·4 per cent. of the animals, in numbers of from 100 to 550.

Genus Trichostrongylus Looss, 1905.

Four species of this genus were seen, namely, *T. axei* (Cobbold, 1879), *T. colubriformis* (Giles, 1892), *T. vitrinus* Looss, 1905, and *T. longispicularis* Gordon, 1933.

T. axei (*Cobbold,* 1879).

Although present to the extent of 75·2 per cent., *T. axei* occurred for the most part in small to moderate numbers. 32·6 per cent. of the infested animals had less than 1,000 worms each and 25·2 per cent. from 1,000 to 5,000 worms respectively. Four animals, 7 to 12 months old, gave an average of 9,620 worms each with a range of 5,000 to 13,500 worms. Of these, an eight months old animal yielded 11,100 worms, whilst 13,500, the highest number recorded, were collected from a yearling.

T. colubriformis (*Giles,* 1892).

This species was found in four animals, 8, 12, 36 and 52 specimens respectively being present.

T. vitrinus Looss, 1905.

Present in one animal, 16 specimens being collected.

T. longispicularis Gordon, 1933.

Seen in 4·4 per cent. of the animals examined and in numbers of from 15 to 220.

Genus Cooperia Ransom, 1907.

Six species of Cooperia were present, namely, *C. oncophora* (Railliet, 1898), *C. curticei* (Railliet, 1893), *C. mcmasteri* Gordon, 1932, *C. pectinata* Ransom, 1907, *C. punctata* (v. Linstow, 1907) and *C. spatulata* Baylis, 1938.

C. oncophora (*Railliet,* 1898).

This species was seen in 20·5 per cent. of the animals. The highest number taken from any animal was 7,500, which came from a six months old heifer. The infections, however, were generally much smaller than this and in 82 per cent. of the positive animals were less than 100 worms per animal.

C. curticei (*Railliet,* 1893).

Present in 5·1 per cent. of the animals, the numbers of this species exceeded 100 for any animal on one occasion only, when a seven months calf yielded 2,500 worms.

C. mcmasteri Gordon, 1932.

Seen in 5·9 per cent. of our animals, the numbers of this species varied from 12 to 550.

C. spatulata Baylis, 1938.

C. spatulata was present in 21·8 per cent. of the animals, but in only relatively small numbers, namely 2 to 250 worms.

C. pectinata Ransom, 1907.

A common helminth in Queensland cattle, *C. pectinata* was taken from 78.2 per cent. of the animals. The highest number seen in any animal, namely 32,500, came from a yearling. In another animal, six months old, 25,600 were present. In the younger animals, 2 to 4 months old, the largest number seen in any animal was 9,700. Of all the positive animals, irrespective of age, 71·8 per cent. yielded less than 5,000 worms each.

C. punctata (*v. Linstow,* 1907).

Also a very common cattle helminth, the prevalence of this species was 72·3 per cent. Three animals, 9 to 15 months old, harboured 13,500, 15,800 and 19,600 worms respectively, the last infection (19,600) being the highest seen. 73·7 per cent. of the positive animals yielded less than 5,000 worms each, whilst in 24·6 per cent. the infections ranged from 5,000 to 10,000 worms.

Genus Nematodirus Ransom, 1907.

N. filicollis (*Rudolphi,* 1802).

This species was present in 17·5 per cent. of the animals. The highest number collected from any animal was 3,000, whilst three other animals gave counts of 1,000 to 2,600 worms. In the remaining animals, the infections were rarely larger than 100 worms.

N. spathiger (*Railliet,* 1896).

Present in one animal only, the infection being represented by ten worms.

Genus Bunostomum Railliet, 1902.

B. phlebotomum (*Railliet,* 1900).

This hookworm was present in 44·5 per cent. of the animals. The majority harboured less than 50 worms each and frequently less than ten. Three animals, nine months, six months and 12 months old, yielded 78, 158 and 264 worms respectively. These were the highest numbers seen.

Genus Strongyloides Grassi, 1879.

S. papillosus (*Wedl.,* 1856).

Present in 57·8 per cent. of the animals, but for the most part in small to moderate numbers (500 to 1,500 worms). One animal, six months old, yielded 32,600 specimens.

Genus Capillaria Zeder, 1800.

Capillaria sp.

Four females of an undetermined species of this genus were collected from the small intestine of one animal.

Genus Bosicola Sandground, 1929.

B. radiatum (*Rudolphi,* 1803).

75·6 per cent. of the animals contained this species. High numbers were met with on several occasions. Fourteen animals of all ages harboured 300 to 500 worms ; six animals, nine to twelve months old, 600 to 700 worms, whilst from two others, twelve months and seven months old, infections of 1,566 and 1,078 worms, respectively, were taken.

Genus Trichuris Roederer, 1761.

A re-examination of the material collected as *T. ovis* (Abildg., 1795) revealed the presence of three species, namely *T. globulosa* (v. Linstow, 1901), *T. parvispiculum* Ortlepp, 1937, and *T. ovis* (Abildg., 1795). Of these, *T. globulosa* appeared most common. *Trichuris* spp. were present in 13·2 per cent. of the animals, the highest number encountered in any animal being 72.

Check List of the Gastro-Intestinal Helminths of Australian Cattle.

Paramphistomum cervi (Schrank, 1790).
P. explanatum (Creplin, 1847).
Cotylophoron cotylophorum (Fischoeder, 1901).
Moniezia expansa (Rudolphi, 1810).
M. benedeni (Moniez, 1879).
Helictometra giardi (Moniez, 1879).
Strongyloides papillosus (Wedl., 1856).
Trichuris ovis (Abildg., 1795).
T. globulosa (v. Linstow, 1901).
T. parvispiculum Ortlepp, 1937.
Capillaria sp.
Bosicola radiatum (Rudolphi, 1813).
Bunostomum phlebotomum (Railliet, 1900).
Trichostrongylus axei (Cobbold, 1879).
T. colubriformis (Giles, 1892).
T. vitrinus Looss, 1905.
T. longispicularis Gordon, 1933.

Cooperia pectinata Ransom, 1907.
C. punctata (v. Linstow, 1907).
C. curticei (Railliet, 1893).
C. oncophora (Railliet, 1896).
C. mcmasteri Gordon, 1932.
C. spatulata Baylis, 1938.
Ostertagia ostertagi (Stiles, 1892).
O. circumcincta (Stadelman, 1894).
O. occidentalis Ransom, 1907.
Hæmonchus contortus (Rudolphi, 1803).
Nematodirus filicollis (Rudolphi, 1802).
N. spathiger (Railliet, 1896).

SUMMARY.

1. In an examination of the alimentary tract of 237 apparently healthy young cattle, helminths were present in 97·13 per cent. Twenty-nine species were found.

2. The most frequently encountered species were *Hæmonchus contortus* (86·7 per cent.), *Ostertagia ostertagi* (75·2 per cent.), *Bosicola radiatum* (75·6 per cent.), *Cooperia pectinata* (78·2 per cent.), *C. punctata* (72·3 per cent.), *Strongyloides papillosus* (57·8 per cent.), and *Bunostomum phlebotomum* (44·5 per cent.).

3. Counts or estimations of the numbers of each species were made in every animal.

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REFERENCES.

Baylis, H. A. (1938). *Ann. Mag. Nat. History*, Ser. 11, **1**, 68.
Ortlepp, R. J. (1937). *Onderstepoort J. Vet. Sc. & Anim. Indus.*, **9**, 1, 91.
Roberts, F. H. S. (1934). "Worm Parasites of Domesticated Animals in Queensland." Animal Health Station, Yeerongpilly, Dept. Agric. & Stock, Q. Pamphlet No. 2.
——— (1935). *Aust. Vet. J.*, **11**, 229.

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